



# Investigation of the solvent-dependent photolysis of a nonnucleoside reverse-transcriptase inhibitor, antiviral agent efavirenz

Maryam A Jordaan and Michael Shapi

## Abstract

This study sought to investigate the solvent-dependency on the photolysis of efavirenz to gain insight into the photo-processes involved. The primary mechanisms were firstly the excited-state intramolecular proton transfer (i.e. phototautomerization), which generated the imidic acid phototautomer observed as  $[M-H]^-$  quasimolecular ion at  $m/z$  314.0070 in the high-performance liquid chromatography–electrospray ionization–time-of-flight mass spectrometry in the negative mode. Secondly, the photoinduced  $\alpha$ -cleavage with the loss of a carbonyl group occurred (i.e. photodecarbonylation) to form the photoproduct at  $m/z$  286.0395. The ultraviolet–visible spectra illustrated a large, hyperchromic, and slight bathochromic effect in both the  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  electronic transitions. The largest bathochromic effect was prevalent in the chloroform solvent, i.e. chloroform ( $\pi^* = 0.58$ ;  $\beta = 0.00$ ;  $\alpha = 0.44$ ) > methanol ( $\pi^* = 0.60$ ;  $\beta = 0.66$ ;  $\alpha = 0.98$ ) > acetonitrile ( $\pi^* = 0.75$ ;  $\beta = 0.40$ ;  $\alpha = 0.19$ ). This is due to the significant interaction of the amino group with the excited carbonyl moiety which is attributed to intramolecular phototautomerization resulting in a larger energy shift of the electronic state. A plausible explanation is due to the hydrogen bond donor ability of the polar methanol and nonpolar chloroform solvents, which stabilized the polarized imidic acid phototautomer by means of hydrogen bonding interactions, as opposed to the aprotic acetonitrile which exhibits no hydrogen bonding interactions. The study would form the basis for further photolytic analyses and syntheses to generate a plethora of novel photoproducts with anti-HIV activity based on the biologically active benzoxazinone framework of efavirenz.

## Keywords

HIV, nonnucleoside reverse-transcriptase inhibitor, AIDS, reverse transcriptase

## Introduction

The onset of HIV/AIDS has led to a despairing situation of disease progression affecting millions of people globally with Africa as its most vulnerable target. Moreover, cancer and HIV infection have been intricately associated since the beginning of the AIDS pandemic.

Although there are currently 27 antiretroviral drugs approved by the United States Food and Drug Administration used to treat HIV/AIDS,<sup>1,2</sup> many of these drugs demonstrate photoreactivity which causes structural changes, subsequently changing the physicochemical properties of the drug. These molecular changes could decrease the therapeutic effects of the antiretroviral drugs and lead to phototoxicity and photoallergy.<sup>3</sup> Efavirenz, Figure 1, a potent antiviral agent, which is characterized by its chromophoric

benzoxazinone framework intermittent with intramolecular hydrogen bonding (H-bonds), can be photoinduced to undergo proton transfer processes known as excited-state intramolecular proton transfer (ESIPT).<sup>4,5</sup> This is mainly due to the presence of lone pair electrons on the nitrogen in efavirenz, which allows for this photoprototropic behavior (i.e. proton migration from C, N, or O centers to N or O centers), referred to as phototautomerization. There is a lack of

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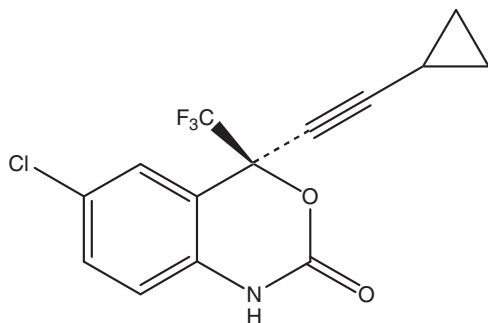


Figure 1. Efavirenz.

understanding of proton transfer reactions, and a direct way to initiate intramolecular proton transfer is by means of photolysis, which in turn allows the opportunity to initiate intramolecular proton transfer reactions in the excited state which can then easily be monitored on a shorter timescale than that of ground-state reactions.<sup>6</sup> Efavirenz can furthermore undergo other photoprocesses, i.e.  $\alpha$ -cleavage reaction, photodecarbonylation, photoisomerism, and photosolvolysis to name a few.<sup>4</sup> Furthermore, in an environmental conscious era where greener synthetic methods are sought, photochemistry has evolved into an alternative synthetic method in contrast to conventional precarious synthetic techniques. It would thus be of great importance to therefore investigate the photolysis of efavirenz to gain insight into the photoprocesses involved. Photochemistry entails the study of molecular transitions in the excited state induced by the absorption of ultraviolet (UV) light. During photochemical reactions, light energy is absorbed promoting an electron from the ground-state orbital ( $S_0$ ) to an excited state orbital ( $S_1$ ). This excitation allows for chemical reactions which cannot take place within traditional ground-state chemistry, and light, thus, serves as a catalyst allowing a range of novel chemical transformations to take place.<sup>7</sup> However, photochemistry remains a neglected research area because of low yields due to competing thermal reactions. The complexity of these photochemical reactions can result in the formation of multiple reaction products of which identification and structural elucidation thereof become cumbersome.<sup>8</sup> These photoproducts can easily be identified by means of high-performance liquid chromatography–electrospray ionization–time-of-flight mass spectrometry (HPLC-ESI-TOFMS), which is used in the structural elucidation of complex mixtures. High-resolution electrospray ionization–time-of-flight mass spectrometry (ESI-TOFMS) offers an added advantage in that it provides information about the molecular formula which is obtained through accurate mass data that subsequently allows the identification of unknown compounds with a

sufficient high resolution, enabling unequivocal assignment of structures in many cases.<sup>9</sup>

Previous studies found in literature focused primarily on the photodegradation of efavirenz by means of sunlight<sup>10</sup> and artificial light sources,<sup>11</sup> as well as the photochemical derivatization of efavirenz in blood plasma.<sup>12</sup> However, most photoreactions occur in solution, and studies conducted failed to address the solvent-dependency on the photolysis of efavirenz. This information is important as the solute interacts with the hydrogen bond acceptor ( $\beta$ ) or donor abilities ( $\alpha$ ) and solvent polarizability ( $\pi^*$ ) of the relevant solvents<sup>5,13,14</sup> producing different reaction mechanisms and products.

This study sought to investigate the solvent-dependency on the photolysis of efavirenz to gain a better understanding of the photoprocesses involved. The qualitative identification of the photoproducts was monitored and assessed by using a combination of HPLC-ESI-TOFMS and UV–visible spectrophotometry. This information would form the basis for further studies whereby photochemistry could be used as a gateway to synthesize and evaluate a plethora of novel photoproducts with anti-HIV activity.

## Materials and methods

### Materials

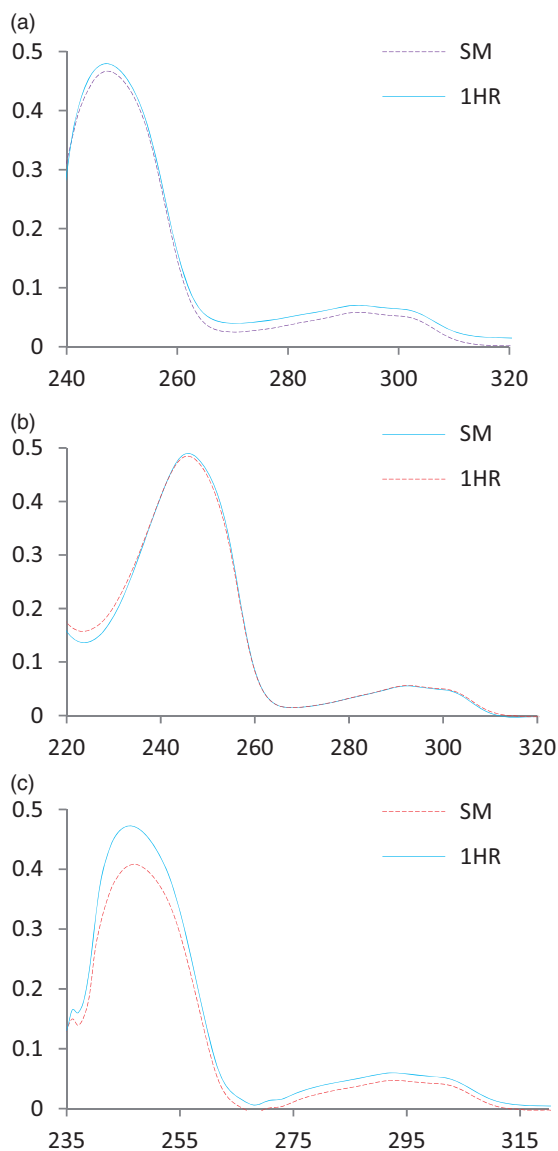
Stocrin<sup>®</sup> tablets (600 mg efavirenz API) (Reg. No. 37/20.2.8/0628) were supplied by Shalom Laboratory Supplies CC, Durban, South Africa. Methanol, acetonitrile, and chloroform were of AR-grade and were purchased from Merck and used without further purification.

### Photochemical procedure

Stocrin<sup>®</sup> tablets were ground with a mortar and pestle, dissolved in petroleum ether, then vacuum filtered, and recrystallized to extract the efavirenz API. Efavirenz (10 mg, 0.03168 mmol) was dissolved in acetonitrile, methanol, or chloroform (10 mL) to form a concentration of 6336 mM. The sample was flushed with nitrogen to remove traces of oxygen, which causes photooxidation and irradiated with UVB (315–280 nm) for 5 h. The UV absorbance was monitored after an hour of exposure to UVB by means of a Perkin Elmer Lambda 35 UV–visible spectrophotometer.

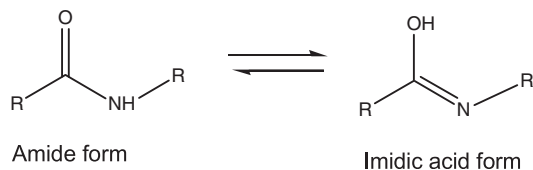
### High-performance liquid chromatography–electrospray ionization–time-of-flight mass spectrometry

HPLC-ESI-TOFMS was used to monitor the reaction progression over 5 hours; 100  $\mu$ L (6336 mM) of the



**Figure 2.** UV absorbance spectrum in (a) methanol (b) acetonitrile (c) chloroform.

UV: ultraviolet; SM: Starting material; HR: Hour.



**Scheme 1.** An example of phototautomerization.

reaction mixture solvent was evaporated off under nitrogen and reconstituted in methanol (1.5 mL) and then analyzed. Chromatography was performed on a Shimadzu UFLC-XR using an Inertsil, C-18,  $250 \times 4.6$  mm (5  $\mu\text{m}$ ) stainless steel column. An isocratic

**Table 1.** Values of the hydrogen bond acceptor ( $\beta$ ), donor abilities ( $\alpha$ ), and solvent polarizability ( $\pi^*$ ) in selected solvents used in the study.

Solvent	$\pi^*$	$\alpha$ (hydrogen donor ability)	$\beta$ (hydrogen bond acceptor ability)	$\lambda_{\text{max}}$ (nm)
Acetonitrile	0.75	0.19	0.40	246, 293
Methanol	0.60	0.98	0.66	248, 293
$\text{CHCl}_3$	0.58	0.44	0.00	247, 293

Source: reproduced with permission from Sancho et al.<sup>13</sup> and Loconto.<sup>14</sup>

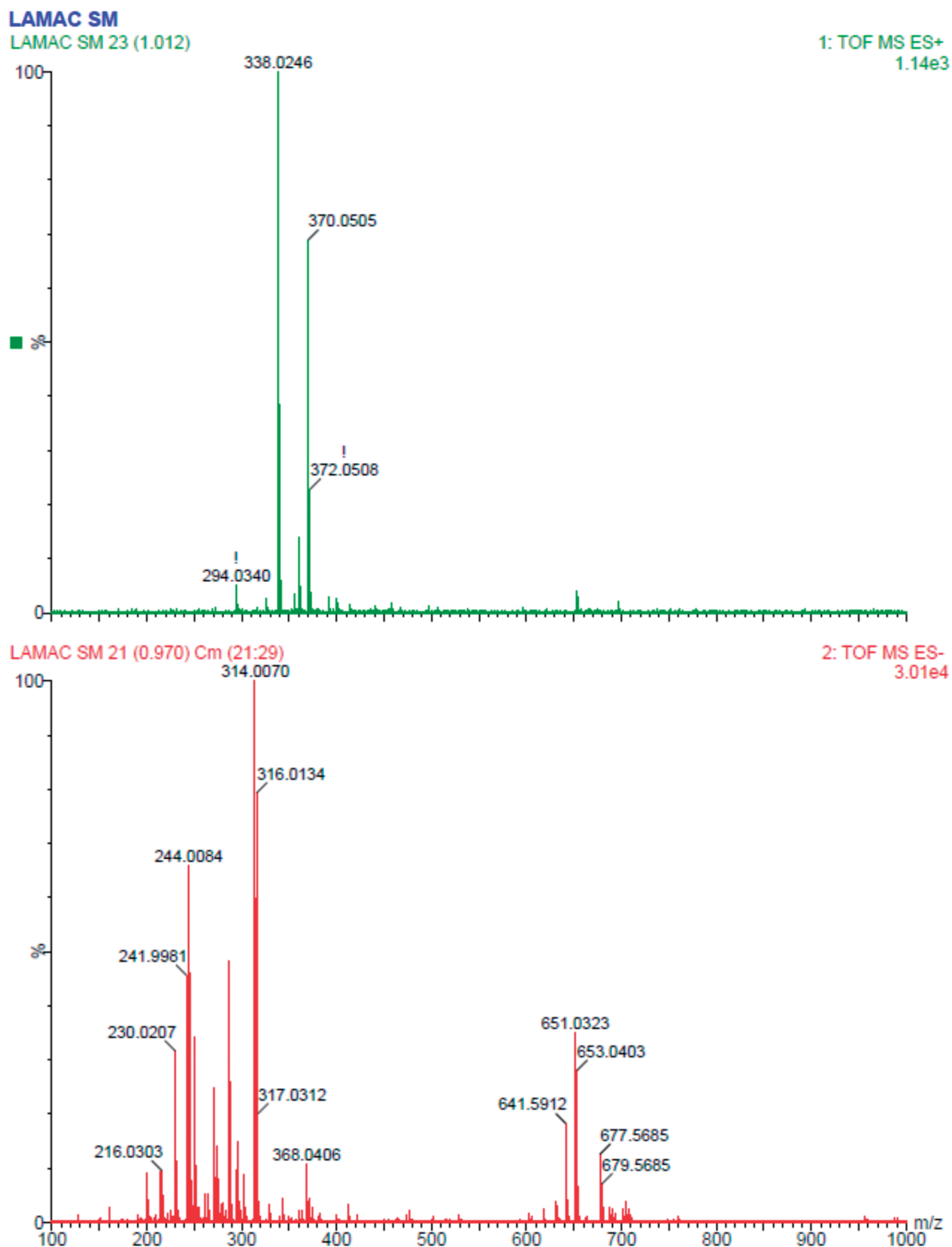
system was set up with the following parameters: 88% MeOH/H<sub>2</sub>O 0.300 mL/min for 15 min and column temperature: 35°C. There is a delay between the Photo Diode Array (PDA) retention time (RT) and the MS RT (the peak has to travel from the PDA into the MS), so the UV RT is slightly lower than requested. The MS parameters were set as follows: capillary voltage: 3000 kV, cone voltage: 20 kV, desolvation temperature: 250°C, source temperature: 120°C, desolvation gas flow: 550 L/h, and cone gas flow: 100 L/h. Both electro-spray positive and negative modes were run.

## Results and discussion

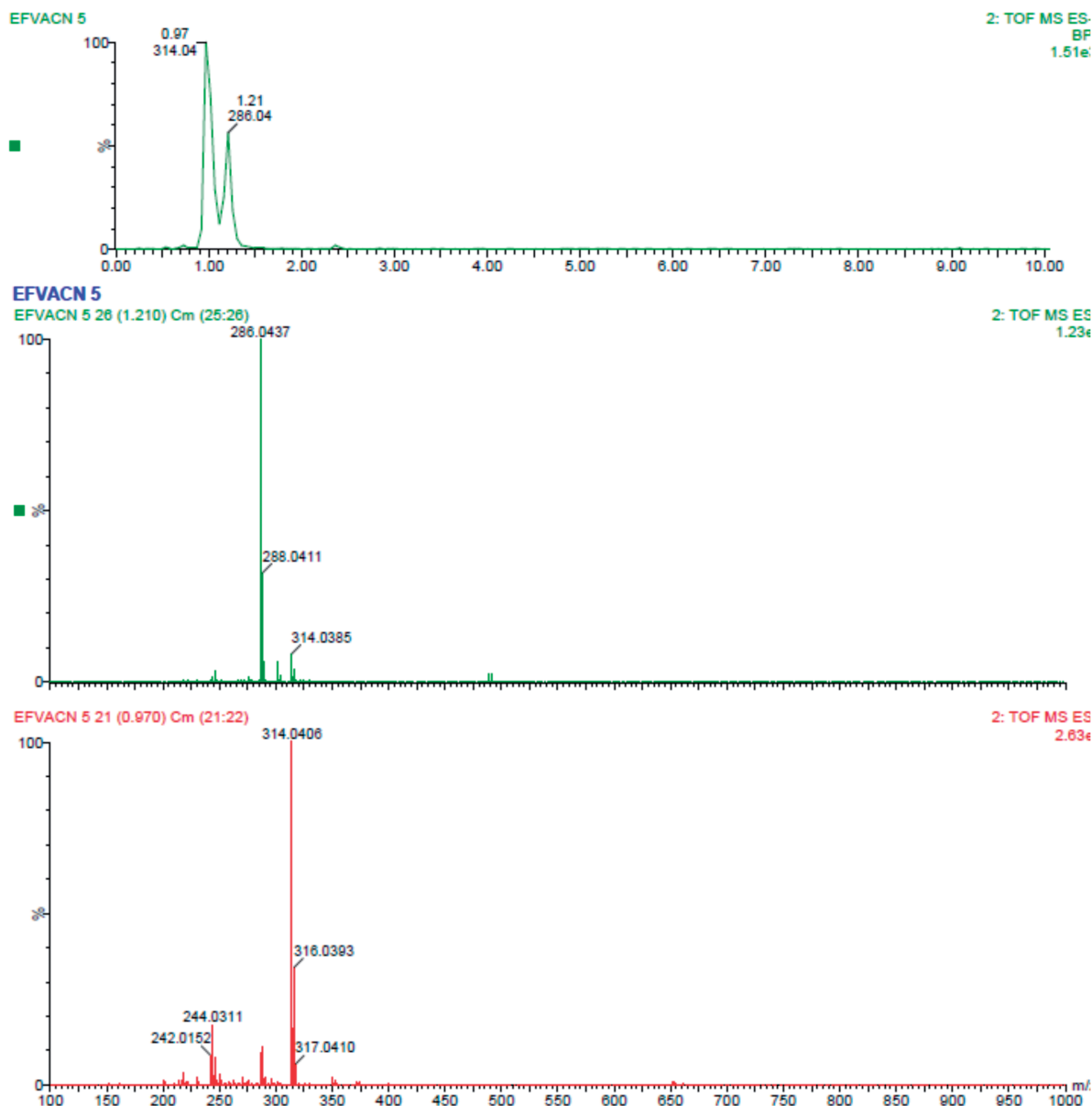
### UV spectroscopic analysis of efavirenz on UVB exposure

The absorption spectra were recorded in methanol, acetonitrile, and chloroform (Figure 2). Two main absorbance maxima were observed for efavirenz at  $\lambda_{\text{max}}$  within a range of 246–248 nm (i.e.  $\pi, \pi^*$  electronic transition) and at  $\lambda_{\text{max}}$  between 292 and 295 nm (i.e.  $n, \pi^*$  electronic transition) depending on the solvent. These observations were consistent with those documented in literature as carbonyl aromatics are characterized by  $\pi, \pi^*$  and  $n, \pi^*$  transition states.<sup>5</sup>

Efavirenz is characterized by its benzoxazinone framework with the presence of an auxochromic, electron-donating amino and electron-accepting carbonyl functionalities; therefore, the main photoreactions that should occur are hydrogen abstraction and  $\alpha$ -cleavage. According to Elguero et al.,<sup>15</sup> the presence of the lone pair electrons on the nitrogen, as seen in efavirenz, would allow for photoprototropic behavior (i.e. proton migration from C, N, or O centers to N or O centers). This atom-transfer results in amide to imidic acid phototautomerization as a potential transformation (Scheme 1). The amide is the electron donor (D), the carbonyl moiety is the electron acceptor (A), and the covalently attached proton attached to the amino group migrates to the neighboring carbonyl atom less than 2 Å away in the electronically excited state.



**Figure 3.** Liquid chromatography time-of-flight mass spectrometry spectrum (LC-QTOFMS) of efavirenz in the positive and negative modes, respectively.

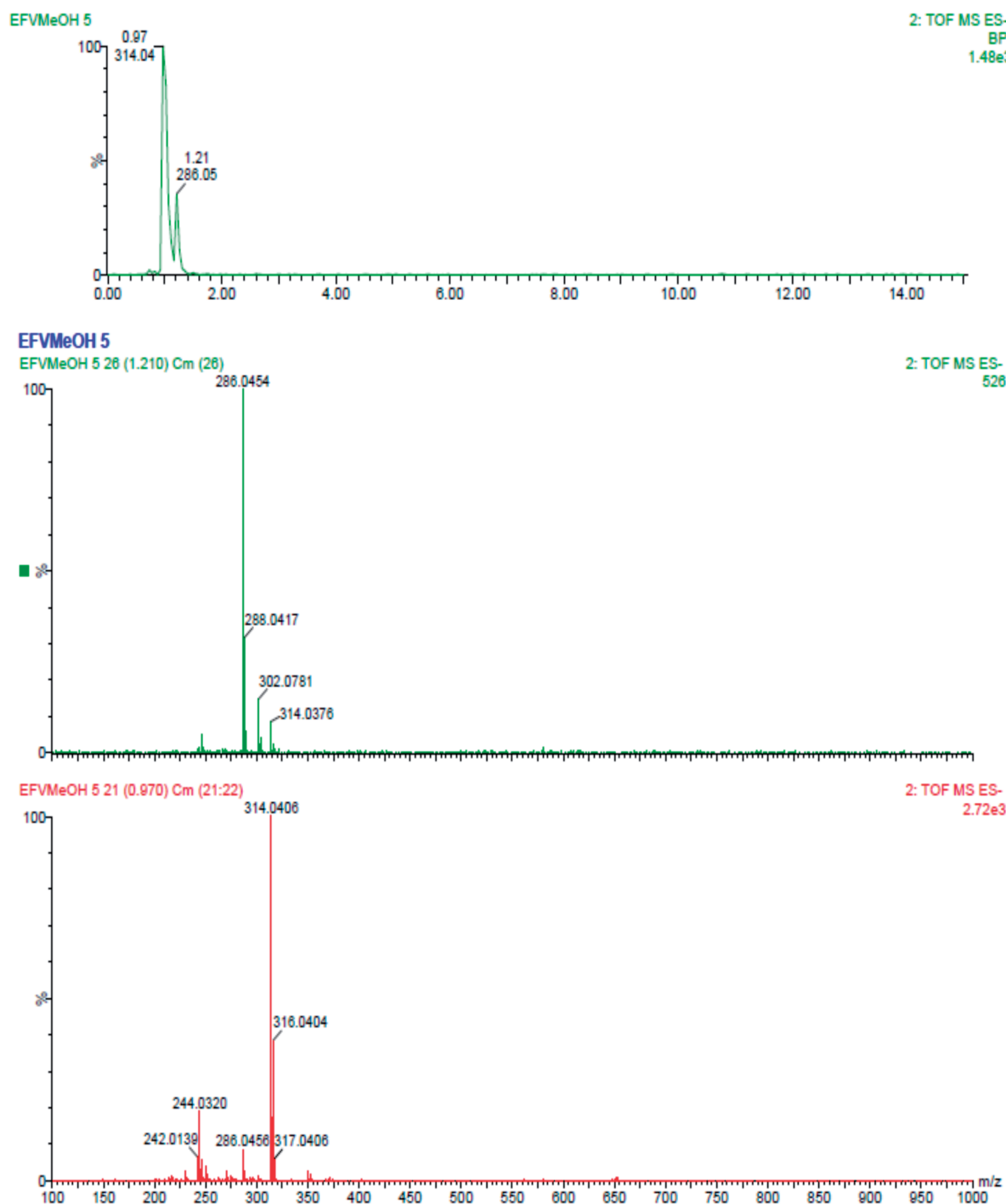


**Figure 4.** LC-QTOFMS (negative mode) of the photolysis of efavirenz in acetonitrile.

Although both tautomers can occur in the ground state on photoexcitation, the phototautomeric pairs can easily be observed by means of UV-visible spectrophotometry.<sup>6</sup>

The UV-visible data of efavirenz are presented in Figure 2 and Table 1. A large hyperchromic and slight bathochromic effect is observed in both bands in the UV-visible spectra on UVB irradiation. The first  $\pi \rightarrow \pi^*$  electronic transition illustrates the excitation of the delocalized benzene electrons. It is well documented that in aromatic molecules containing a hydroxy (OH) or amine (NH) group, the  $n \rightarrow \pi^*$  states induce a hydrogen-transfer process from the chromophore to the solvent in protic solvents.<sup>16</sup> According to

literature, it is also known that the photoexcitation of carbonyls in the presence of amines results in an electron transfer from the hydrogen atom-donating amine to the excited carbonyl chromophore that is red shifted. The primary bond-forming step in this transformation is the addition of a hydrogen atom to the C=O chromophore to form a new O—H bond. Formation of a new O—H bond can, therefore, occur by way of an electron transfer followed by a proton transfer (Scheme 3).<sup>17</sup> The reduction occurs from both  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  states. It has been disputed that these types of molecules in the excited state, being more polar than in the ground state, should be stabilized more than in the ground state by a polar or polarizable medium.

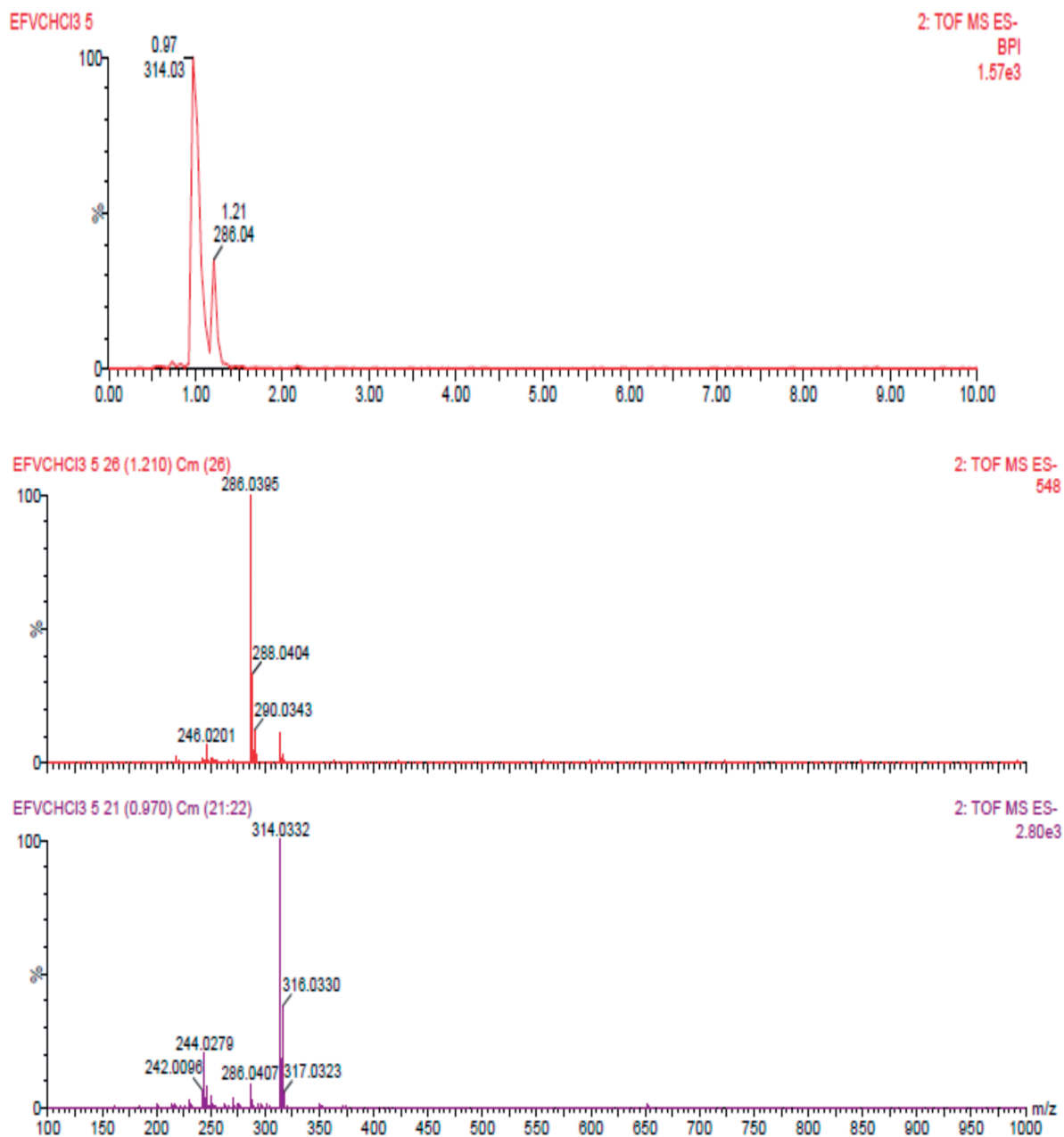


**Figure 5.** LC-QTOFMS (negative mode) of the photolysis of efavirenz in MeOH.

The second  $n \rightarrow \pi^*$  electronic transition of efavirenz indicated an internal proton transfer from the adjacent  $-\text{NH}$  group to the  $\text{C}=\text{O}$  group and yields the imidic anion in the excited state as the acidity of aromatic amines and alcohols is much higher in the excited state than that in the ground state.

The largest bathochromic effect was noticed in the chloroform solvent, i.e. chloroform ( $\pi^* = 0.58$ ;

$\beta = 0.00$ ;  $\alpha = 0.44$ ) > methanol ( $\pi^* = 0.60$ ;  $\beta = 0.66$ ;  $\alpha = 0.98$ ) > acetonitrile ( $\pi^* = 0.75$ ;  $\beta = 0.40$ ;  $\alpha = 0.19$ ). This spectroscopic observation is due to the significant interaction of the amino group with the excited carbonyl moiety and can be attributed to intramolecular phototautomerization, which results in a larger energy shift of the electronic state. A plausible explanation for these observations is that the

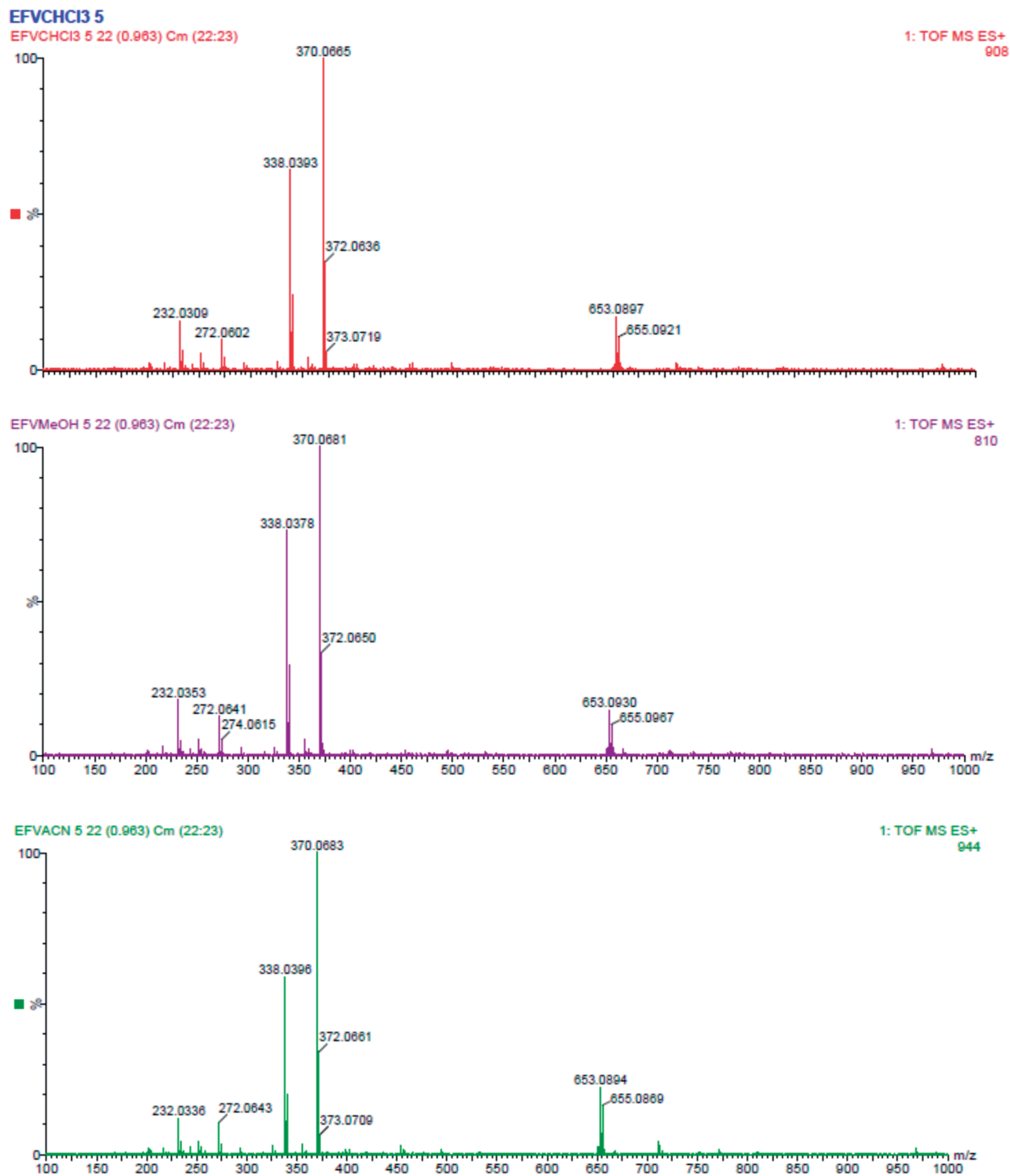


**Figure 6.** LC-QTOFMS (negative mode) of the photolysis of efavirenz in chloroform.

hydrogen bond donor ability of the polar methanol and nonpolar chloroform solvents has resulted in the stabilization of the polarized imidic acid phototautomer by means of hydrogen bonding interactions, as opposed to the aprotic acetonitrile, which exhibits no hydrogen bonding interactions. Conversion to imidic acid is a higher energy transformation in comparison to carbon protonation and results in a large energy shift of the electronic state as observed in the spectra.<sup>6</sup>

#### *Structural identification of photoproducts and intermediates by HPLC-ESI-TOFMS*

HPLC-ESI-TOFMS spectra were analyzed in both the positive and negative modes (Figures 3 to 7), and data are presented in Table 2. The efavirenz  $[M-H]^-$  quasi-molecular ion at  $m/z$  314.0070 was prevalent in the negative mode at an RT of 1.02 min. The intramolecular ground-state proton transfer or tautomerization at room temperature was responsible for the observed



**Figure 7.** MS spectra (positive mode) on the photolysis of efavirenz in chloroform, methanol, and acetonitrile, respectively. MS: mass spectrometry.

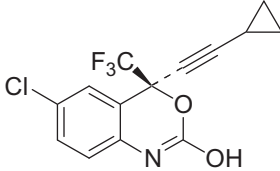
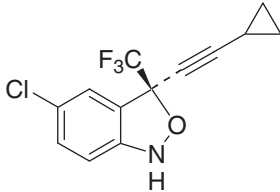
result (Figure 6). Additionally, the amide centers are prone to the formation of intermolecular hydrogen bonds with the solvent or with the compound itself to form dimers at  $m/z$  679. In contrast to our observations, Saira et al.<sup>18</sup> used liquid chromatography–mass spectrometry (LC-MS) to analyze efavirenz and an impurity, and they found that efavirenz exhibited the

$[M + 1]^+$  quasimolecular ion at  $m/z = 316.0$  Da in the positive mode.

UV irradiation of efavirenz in methanol, acetonitrile, and chloroform produced the efavirenz  $[M-H]^-$  quasimolecular ion at RT of 0.97 min with  $m/z$  314.0070 (Figures 4 to 6). In general, the analysis of amides by means of ESI-LCMS is predominantly

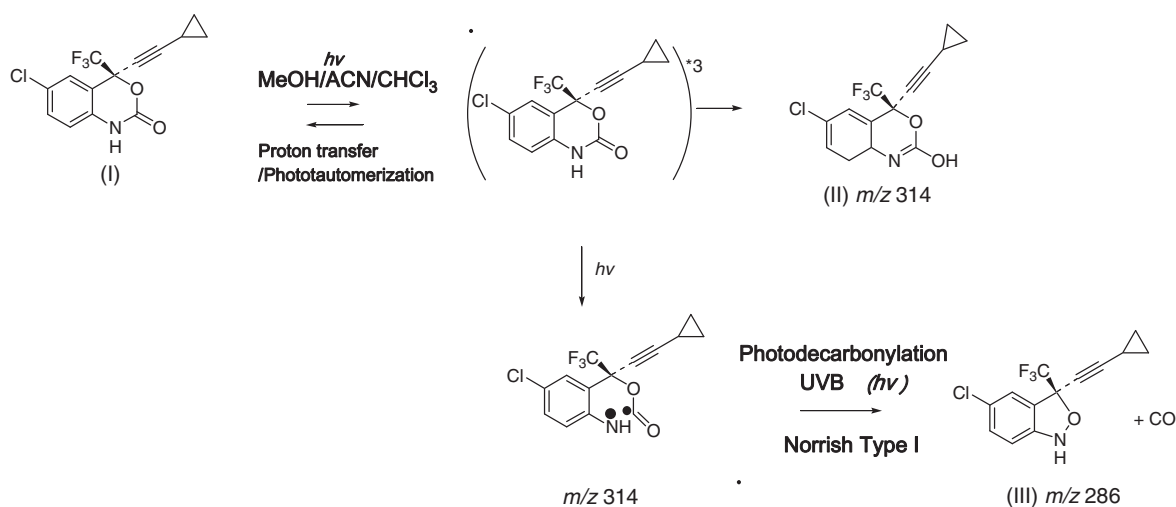


**Table 2.** Accurate mass measurements obtained from the LC-TOFMS spectra of the efavirenz (de)protonated phototransformation products identified.

Name	Retention time, RT (min)	Accurate mass <sup>a</sup> (Da) +	Quasimolecular ion ( <i>m/z</i> )	Molecular formulae	Postulated chemical structure
I	1.01	315.0274	[M-H] <sup>-</sup> : 314.0070	C <sub>14</sub> H <sub>9</sub> ClF <sub>3</sub> NO <sub>2</sub>	
III	1.21	287.0325	[M-H] <sup>-</sup> : 286.0395	C <sub>13</sub> H <sub>9</sub> ClF <sub>3</sub> NO	

LC-TOFMS: liquid chromatography–time-of-flight mass spectrometry.

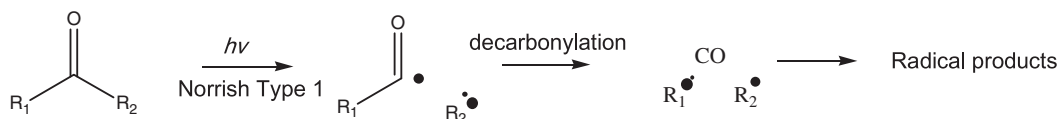
<sup>a</sup>Calculations are based on the following exact masses: C, 12.000000; H, 1.007825; Br, 78.918348; Cl, 34.968855; F, 18.998405; N, 14.003074; and I, 126.904352.<sup>22</sup>

**Scheme 2.** Phototautomerization and photodecarbonylation of efavirenz.

done in the positive mode as amide centers are prone to the formation of hydrogen bonds and can therefore be readily protonated.<sup>19</sup> Although amides are good proton acceptors, no positive [M+H]<sup>+</sup> quasimolecular ion (Figure 7) was observed, suggesting that the amide exists primarily as the imidic anion (Scheme 1). This can be explained by the benzoxazinone excited state, which has considerable charge-transfer character. Electron transfer subsequently occurs from the amine to the benzoxazinone triplet, and subsequent hydrogen abstraction prevails leading to the proposed imidic acid phototautomer (II) (Scheme 2). Deprotonation is now

possible *via* the OH of the imidic acid tautomer, resulting in the observable resonance-stabilized anions. Efavirenz, therefore, ionizes readily in the negative mode due to its high acidity in the excited state, which was considerably red-shifted via intramolecular proton transfer, as observed in the UV spectra (Figure 2). This is consistent with literature as aromatic amines epitomize classic examples of enhanced acidity in the excited state, and this is referred to as a “photo-acid”.<sup>20</sup>

Furthermore, the main photoproduct observed is 28 amu less than its main [M-H]<sup>-</sup> ion at RT of



**Scheme 3.** Photodecarbonylation reaction. Source: reproduced with permission from Moss et al.<sup>21</sup>

1.21 min with  $m/z$  286.0395 in the negative mode. This observation is indicative of a loss of the CO group and corresponds to a photodecarbonylation reaction (Scheme 2). The main fragment ion at  $m/z$  246 corresponds to a loss of 40 amu from the photoproduct. Photodecarbonylation is a typical example of the Norrish Type I photoreaction, which occurs after production of the diradical on photoinduced  $\alpha$ -cleavage; CO is then eliminated (Scheme 3)<sup>21</sup>. The polar carbonyl functionality exhibits  $\alpha$ -cleavage as the main photochemical reaction with stabilization of  $\alpha$ -carbanion. These results are in contrast to the results obtained by Matthew et al.<sup>12</sup> who deduced that the major photolysis product of efavirenz in blood plasma was 6-chloroquinoline substituted in positions 2 and 4 with cyclopropyl and trifluoromethyl groups, which was observed as a single ion at  $m/z$  272. This was 44 units less than the  $[M+H]^+$  peak for efavirenz which was observed at  $m/z$  316 in the positive mode and corresponded to the loss of CO<sub>2</sub> from the molecule. This indicated that the product was formed by the photodecarboxylation of efavirenz.

## Conclusions

An investigation into the solvent-dependency of the photolysis of efavirenz is provided for the first time. The observed UV-visible and HPLC-ESI-TOFMS spectra demonstrated that ES IPT or phototautomerization followed by photodecarbonylation were the main photoprocesses that had taken place. UV-visible data were used to interpret the microscopic effect of the respective solvents, i.e. methanol, acetonitrile, and chloroform, on the phototautomerization of efavirenz. It was concluded that the largest bathochromic effect was noticed in the chloroform solvent, i.e. chloroform ( $\pi^* = 0.58$ ;  $\beta = 0.00$ ;  $\alpha = 0.44$ ) > methanol ( $\pi^* = 0.60$ ;  $\beta = 0.66$ ;  $\alpha = 0.98$ ) > acetonitrile ( $\pi^* = 0.75$ ;  $\beta = 0.40$ ;  $\alpha = 0.19$ ). This spectroscopic observation is due to the significant interaction of the amino group with the excited carbonyl moiety and can be attributed to intramolecular phototautomerization, which results in a larger energy shift of the electronic state. A plausible explanation for these observations is that the hydrogen bond donor ability of the polar methanol and nonpolar chloroform solvents resulted in the stabilization of the polarized imidic acid phototautomer by means of hydrogen bonding interactions as opposed to the

aprotic acetonitrile which exhibits no hydrogen bonding interactions. From the HPLC-ESI-TOFMS data, it was evident that the  $[M-H]^-$  ions generated from the phototautomerization of efavirenz were observed in the negative mode at  $m/z$  314.0070 due to the high acidity of efavirenz that did not ionize well in the positive mode. Photoinduced  $\alpha$ -cleavage followed with the loss of a carbonyl group to form the photoproduct at  $m/z$  286.0395. This photodecarbonylation reaction was in contrast to the information found in literature which predicted a photodecarboxylation reaction on the derivatization of efavirenz in blood plasma. The information from this study would form the basis for further photolytic analyses and syntheses which could be used to generate a plethora of novel photoproducts with anti-HIV activity based on the biologically active benzoxazinone framework of efavirenz.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors are grateful to the National Research Foundation (NRF grant UID: 102425) and Mangosuthu University of Technology for financial support.

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