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Research Article

ACID DEGRADATION STUDIES OF ABACAVIR SULFATE BY LC-MS/MS

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ABSTRACT

Forced degradation or accelerated degradation is a process whereby the natural degradation rate of a product or material is increased by the application of an additional stress. Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Abacavir sulphate was subjected to forced degradation under the conditions of hydrolysis (acid, alkali and neutral) as prescribed by ICH. Five degradation products were formed and their separation was accomplished on Waters Xterra C_{18} (250mm×4.6mm,5µm) column using 20mM ammonium acetate:acetonitrile as a mobile phase by liquid chromatography. the degradation products were characterized by LC-MS/MS and The m/z values of degraded products were found to be 152,151,192 and 191 respectively. This method is highsensitive, accurate, Rapid and has been validated.

Keywords: Abacavir sulphate, Forced degradation studies, acid degradation studies.

1. INTRODUCTION

Abacavir sulphate (1S, cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9yl]-2-cyclopentene-1 methanol sulphate (Fig. 1) is one of the nucleoside reverse transcriptase inhibitors (NRTIs) used to treat acquired immunodeficiency syndrome(AIDS) caused by human immunodeficiency virus (HIV),the activity of the drug is caused by the intracellularly formed carbovir triphosphate. Formulations of abacavir sulphate are sold under the trade name of Ziagen for oral administration. Theirstability is greatly influenced by varied environmental condition ssuch as temperature, light and humidity[1]. A very few reports are available in the literature on degradation of abacavir sulphate. A few HPLC and LC–MS methods for the determination of abacavir sulphate in plasma, human serum and other biological matrices were reported[2–4]. Simultaneous determination of abacavir sulphate and other antiretroviral in pharmaceutical dosages was also carried out[5–11]. Seshachalam et al. have studied its forced degradation by HPLC[12]. However, neither the extent of degradation nor the characterization of degradation products was reported. The monograph published by World Health Organization (WHO) reported six impurities, but their classification into process related and degradation products was not described [13]. The complete acid degradation profile of abacavir and the mechanism of its degradation products are not yet reported in the literature. Mass spectrometry (MSn) and LC coupled with mass spectrometry (LC–MS, LC–MS/MS) are becoming the most versatile techniques for characterization of pharmaceutical degradation products and impurity profiling[14]. The present work describes the acid degradation behaviour of abacavir sulphate under hydrolysis (acid, base and neutral)and optimization of liquid

chromatographic conditions to separate the drug and its acid degradation products on a reversed-phase C18 column. Method validation, characterization of degradation products and fragmentation pathways of acid degradant using LC–MS/MS were proposed.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Abacavir sulphate (99% purity) was a gift sample from a local manufacturing unit in Hyderabad, India. Glass-distilled and deionised water (Nanopure, Bransted, USA) was used. HPLC grade acetonitrile was purchased from Rankem (Mumbai, India). Analytical reagent grade sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from S.D. Fine Chemicals (Mumbai, India).

2.2. Instrumentation

The HPLC system consisting of two LC-20AD pumps, SPDM20A diode array detector, SIL-20AC auto sampler, DGU-20A3 degasser, and CBM-20A system controller (all from Shimadzu, Kyoto, Japan) were used. A reverse phase Waters XTerra C18 column (250mm×4.6mm i.d., 5µm) was used for separation of all the compounds. The chromatographic data were recorded using an HP-Vectra (Hewlett Packed, Waldron, Germany) computer system with LCsolutions data acquiring software (Shimadzu, Kyoto, Japan). LC–MS/MS was performed by Agilent1100 series online ion trap MSD mass spectrometer with APCI source in positive mode equipped with an autosampler (G1329A), and diode array detector (G1315B) (all from Agilent technologies, Waldbronn, Germany). The data was acquired and processed using LC/MSD trap software 4.2 (Bruker, Waldbronn, Germany). The high resolution mass spectrometry (HRMS) data was acquired using a Q-TOF mass spectrometer (QSTAR XL, Applied Bio systems/MDS Sciex, USA), equipped with an ESI source. The data acquisition was under the control of Analyst QS software.

2.3. Forced degradation procedure

Forced degradation of abacavir sulphate was carried out according to ICH guidelines Q1A (R2)[15]. About 10.0mg of abacavir sulphate was subjected to forced degradation under acidic, basic and neutral conditions by refluxing with 10.0mL of 1N HCl, 1N NaOH and water at 80 ◦C for 24, 48 and 72 h respectively. After completion of hydrolysis all the collected samples were kept in refrigerator at 5 ◦C. The degradation products of acid and base hydrolysis were neutralized with sodium hydroxide and hydrochloric acid respectively. The samples were further diluted to 10 times with de-ionized water. All the samples were filtered through 0.22µm membrane filter before HPLC analysis.

2.4. Chromatographic conditions

All the samples were analyzed by HPLC on a Waters XTerra, C18 column using 20mM aqueous ammonium acetate: acetonitrile as a mobile phase in gradient elution at a flow rate of 1.0 mL/min at ambient temperature. The detection was carried out by photodiode array detector (PDA) at 220 nm.

2.5. Mass spectrometric conditions

The mass spectra were recorded in atmospheric pressure chemical ionization (APCI) in positive mode of detection. Nitrogen was the nebulizer and curtain gas. Collision-induced dissociation was achieved by helium as a collision gas. The ion source conditions were set as follows: dry temperature, 325 ◦C; nebulizer gas, 60 psi; dry gas, 5.0 L/min; capillary exit, 113.5 V; capillary current, 81.787 nA; corona current, 4000 nA; electro multiplier voltage, 2100 V; vaporizer temperature, 400 ◦C and dwell time, 200 ms. The HRMS data was acquired using a Q-TOF mass spectrometer equipped with an ESI source. The typical source conditions were: capillary voltage, 5.00 kV (positive mode 4 kV); declustering potential, 60 V; focusing potential, 220 V; declustering potential- 2, 10 V; resolution 10,000 (full-width half-maximum). Ultra-high pure nitrogen was used as a curtain and collision gas, whereas zero air was used as a nebulizer. For the collision-induced dissociation (CID) experiments, the precursor ion was selected using the quadrupole analyzer and TOF analyzer analyzed the product ions.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

During the optimization process, preliminary experiments were carried out on Waters XTerra, C18 column (250mm×4.6mm,5µm) using water: acetonitrile (90:10, v/v) as a mobile phase. The peaks corresponding to degradation products did not resolve completely and tailing was noticed. To get acceptable separation between the drug and its degradation products, ammonium acetate buffer was used instead of water. Aqueous ammonium acetate buffer (20 mM): acetonitrile in gradient elution mode was successful for

separation of the abacavir sulphate and its degradation products. The optimized gradient elution programme is shown in Table 1. The flow rate was 1.0 mL/min and detection wavelength was 220 nm.The runtime was 25.0 min. The optimized chromatographic conditions were used for separation of abacavir and its degradation products. The method was validated with respect to the parameters outlined in ICH guidelines Q1A (R2) and extended to LC–MS/MS.

3.2. VALIDATION

3.2.1. Specificity

Specificity is the ability of the analytical method to measure the analyte concentration accurately in presence of all the potential impurities. The specificity was determined by subjecting API to stress under hydrolysis. All acid degradation products were well separated from the API and the method was found to be specific.

3.2.2. Linearity

Good linearity was observed in the concentration range 0.5–10.0µg/mL of API. The data was subjected to statistical analysis using a linear regression model; the linear regression equation and correlation coefficient (r2) were Y = 55,868X + 15,285, >0.9960 respectively. The results have indicated a good linearity. The limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield a signal-to noise ratio of 3 for LOD and 10 for LOQ respectively. The LOD and LOQ values were found to be 20 and 60 ng/mL respectively.

3.2.3. Accuracy and precision

Intra- and inter-day precision and accuracy was assessed using three quality control samples. Five replicates were analyzed everyday to determine the intra-day accuracy and precision. The procedure was repeated three times over 3 days in order to determine the inter-day accuracy and precision. Good precision and accuracy was observed. The intra- and inter-day precision data is given inTable2.

3.3. Degradation behaviour under hydrolysis

Fig. 2 shows the typical chromatograms of the degradation products formed under hydrolytic conditions. The chromatographic parameters i.e., retention times, resolution, tailing factor and peak purity were determined and given in Table 3. Under acid hydrolysis, the drug degraded completely resulting in five degradation products (X1–X5). However, no degradation was observed under base and neutral hydrolysis. The degradation Products of acid hydrolysis were analyzed by LC–MS and the chromatographic data is shown intable3.

3.4. Kinetics of degradation

To perform kinetic studies the temperature of acid hydrolysis was altered to 65 and 50 ◦C. A series of stressed samples were collected at 0.5, 1.0, 2.0, 3.0, 6.0, 9.0, and 12.0 h of acid hydrolysis and analyzed by HPLC. Then the chromatographic data revealed that only two degradation products (X2 and X5) were formed, where X5 as major product. At the selected temperature the degradation process of abacavir sulphate could be described by apparent first-order kinetics. At temperatures 50 and 65 ◦C, strict straight line behaviour (correlation coefficient, r2 > 0.999) was observed. It confirms the degradation process was first-order kinetic model. From the slopes of the straight lines it was possible to calculate the apparent first-order degradation rate constant k, half-life (t1/2), and time required for 90% degradation (t90) at each temperature was shown in table 4. The rate constant was increased from0.0064 h−1 at 50 ◦C to 0.0474 h−1 at 65 ◦C.

3.5. MS^n study of abacavir sulphate

The MS^n spectra of ABC are shown in Fig 3. At lower collision energy 0.2 mA, protonation of the drug took place and the molecular ion peak atm/z 287 was observed. At high collision energy 0.45 mA, the elimination of cyclopentenyl moiety from 9-N atom of purine ring was observed by the formation of corresponding protonatedadenine ion at m/z 191. Similar results could be found in the literature for nucleo bases and related synthetic substrates[16, 17]. The MS3 studies at collision energy 0.55 mA, revealed that the ion at m/z 191 underwent further fragmentation gave three product ions atm/z 151, 164 and 174. The former ion was formed due to the Loss of cyclopropene [18], whereas the later ions were generated by the loss of neutral molecules viz., HCN and NH3 respectively[19].

3.6 Characterization of acid degraded products

Abacavir sulphate, on acid hydrolysis yielded five degradation products. Table 5gives the m/z values of the degradants and its fragmentation ions. It starts with the cleavage of bond between cyclopentenyl moiety and 9-N atom of purine ring resulted to degradant A5 at

m/z 191.1442 Da. The combination of fragment ions 134 and 58 resulted in A5.The same was supported even by their elemental composition, calculated from accurate masses, as C8H11N6 +. The molecular ion peak A2 (151.0679 Da) formed by hydrolysis at cyclopropyl ring from A5 leads to the formation of 2-amino adenine. The fragment ion atm/z 134 formed from A2 by the loss of 17amu revealed that the precursor ion contains amine group. It followed same fragmentation pattern as the degradation product A1. In case of A3, the experimental m/z value was 192.0879 Da and its suggested elemental composition was C8H10N5O+. Its formation may be best explained by an attack of water molecule on the NH2 group of purine moiety followed by the elimination of NH3 [14, 15]. The CID MS2 ofm/z 192 ion gave m/z 135 (loss of cyclopropyl amine), and m/z58 (cyclopropyl amine cation) ions. The accurate mass measurement data and the formation of m/z 135 and m/z 58 ions may also supports the structure of A3. Another degradation product A1(152.0566 Da) formed by hydrolysis at cyclopropyl ring from A3leads to the formation of 6-amino-3H-purin-2(9H)-one. Its suggested chemical formula was C5H6N5O+. The identical fragment ions at m/z 135 and 110 revealed that the degradant A1 was the primary degradant pair ofA3. According to nitrogen rule the precursor ion (A1) at m/z 152 should have odd number of nitrogen atoms. The peak A4 at retention time 7.9 min was not detected by LC–MS, possibly due to poor ionizability of the molecule.

CONCLUSION

A rapid, sensitive and accurate LC-MS/MS method was developed and validated for analysing acid degraded products of abacavir sulphate. The degradation behaviour of abacavir sulphate under hydrolysis (acid, base and neutral) was characterised and fragmentation pathways were proposed based on LC-MS/MS data. And the degradation behaviour of abacavir sulphate was best explained by kinetic studies. The established method was successfully applied to determine the acid degraded products of abacavir sulphate.

Table 1: Gradient elution programme

Time	Mobile phase	
	A (%)	**B (%)**
0.00	95.0	5.0
10.00	85.0	15.0
15.00	40.0	60.0
20.00	10.0	90.0
25.00	95.0	5.0

A: ammonium acetate buffer: B: acetonitrile

Table 2: intra- and inter-day precision data

Concentration (µg/ml)	Measured concentration (µg/ml)±RSD (%)	
	Intra-day	Inter-day
2.0	2.301 ± 1.201	2.300 ± 1.3001
5.0	5.2010 ± 0.599	5.2019 ± 0.6
20.0	20.01 ± 0.302	20.031 ± 0.3021

RSD= Relative standard deviation.

Table 3: peak purity and retention data of degradation products

Degradation products	Retention time (min)	P.P.I	S.P.T	m/z (amu)	Tailing factor(T_f)	resolution
X1	4.1	0.9993	0.9965	152.0	1.22	0.0
X2	4.9	0.9999	0.9967	151.0	1.19	3.5
X3	6.9	0.9938	0.9338	192.0	1.11	6.5
X4	7.9	0.9999	0.9983	ND	1.20	2.7
X5	12.1	0.9999	0.9993	191.2	1.17	12.7

P.P.I= peak purity index, SPT= single point threshold, ND= not detected

Table 4: Kinetic study of abacavir under acid hydrolysis

Temperature (◦C)	K_{obs} (h^{-10})	t1/2 (h)	t_{90} (h)
50	0.0064	108.28	359.84
65	0.0474	14.60	48.58

K= degradation rate constant, $t_{1/2}$= half-life

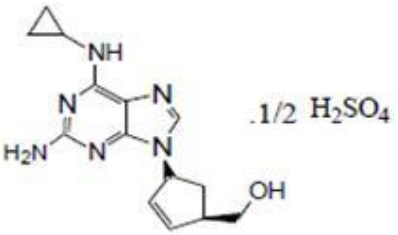


Fig. 1: chemical structure of abacavir sulphate

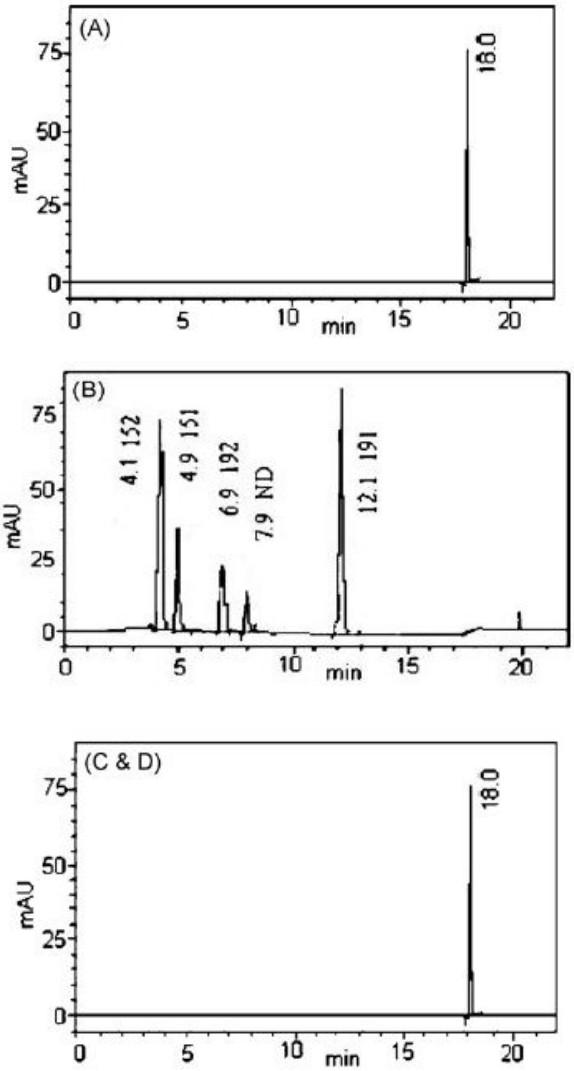


Fig. 2: typical chromatograms of abacavir sulphate(A) and its degradation products under acid hydrolysis(B), base hydrolysis(C), neutral analysis (D)

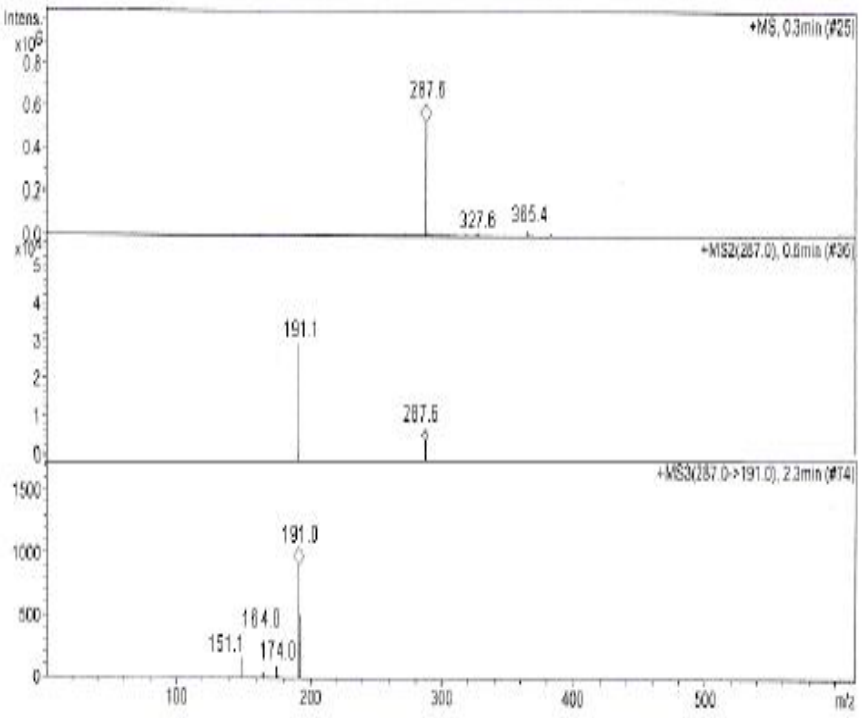
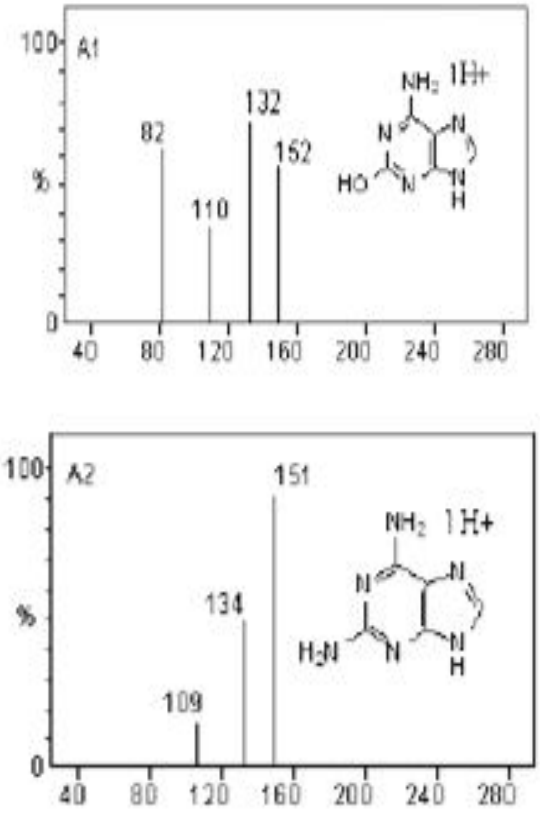


Fig. 3: MS^n fragmentation mass spectra of abacavir sulphate



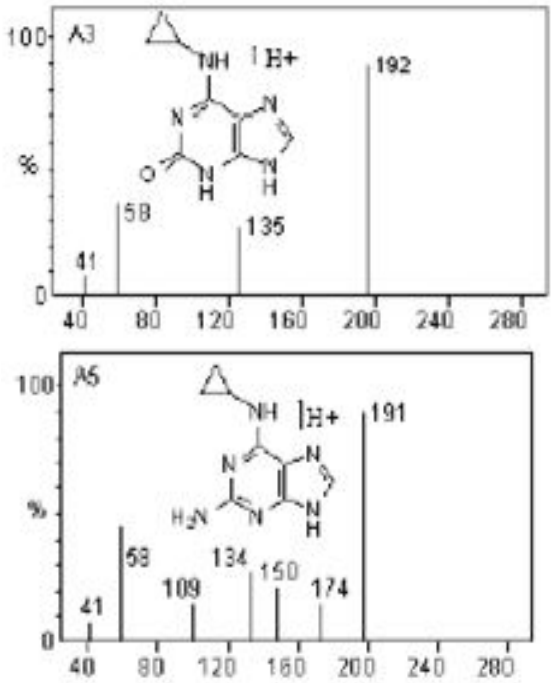


Fig. 4: Mass spectra of the degradation products of abacavir sulphate under acid hydrolysis

REFERENCES

1. Gallant. JE. Initial therapy of HIV infection. J Clin Virol.2002;25:317–333.
2. Stefania N, Alessio B, Giuseppe I and Pasaqale. N. Simultaneous determination of 16 anti HIV drugs in human plasma by high-performance liquid chromatography. J Chromatogr B. 2006;831:258–266.
3. Elisabete AP, Gustavo AM and Marina FMT. Determination of antiretroviralagents in human serum by capillary electrophoresis. J Chromatogr A. 2005;1091:169–176.
4. Verweji-van CPWGMW, Aarnoutse RE and Burger DM. Simultaneous determinationOf the HIV nucleoside analogue reverse transcriptase inhibitorslamivudine, didanosine, stavudine, zidovudine and abacavir in human plasmaby reversed phase high performance liquid chromatography. J Chromatogr. B. 2005;816:121–129.
5. Naser LR, Richard RT, Kashuba DM. Simultaneous determination of six HIVnucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet absorbance detection. J Chromatogr B. 2003;791: 137–147.
6. Joshua RR and Caroline GM. High-performance liquid chromatographic assay forabacavir and its two major metabolites in human urine and cerebrospinal fluid. J Chromatogr B. 2001;762:165–173.
7. Aymard G, Legrand M, Trichereau N and Diquet B. Determination of twelveantiretroviral agents in human plasma sample using reversed-phase highperformanceliquid chromatography. J Chromatogr B. 2000;744:227–240.
8. Agnes IV, Rolf WS, Richard MWH, Jos HB. Quantitative determinationof abacavir (1592U89), a novel nucleoside reverse transcriptase inhibitor, inhuman plasma using isocratic reversed-phase high-performance liquid chromatographywith ultraviolet detection. J Chromatogr B. 1999;736:123–128.
9. Yalcin O, Ayhan S and Sibel AO. Simple and reliable HPLC method of abacavirdetermination in pharmaceuticals, human serum and

drug dissolution studiesfrom tablets. J Liq Chromatogr Relat Technol. 2005;28:423–437.
10. Salut MF, Pilar M, Cecilia FL and Eduardo LM. Determination of abacavir in human plasma by high performance liquid chromatography with ultravioletdetection and the analytical error function. Biomed Chromatogr. 2004;18:862–865.
11. Severine C, Dimitri S, Mikael L and Alian P. Development and validation of a liquid chromatographic/tandem mass spectrometric assay for the quantitation of nucleoside HIV reverse transcriptase inhibitors in biological matrices. J Mass Spectrom. 2005;40: 9–18.
12. Seshachalam U, Haribabu B and Chandrasekhar KB. Development and validationof a reverse-phase liquid chromatographic method for assay and relatedsubstances of abacavir sulphate. J Sep Sci. 2007;30:28–34.
13. http//www.who.int/medicines/publications/pharmacopia/index.html.
14. Wu Y. The use of liquid chromatography–mass spectrometry for the identificationof drug degradation products in pharmaceutical formulations. Biomed Chromatogr. 2000;14:384–396.
15. International Conference on Harmonization, IFPMA, ICH, Geneva, 2003;Q1A(R2).
16. Bedse G, Kumar V and Singh S. Study of forced decomposition behavior of lamivudineusing LC, LC–MS/TOF and MSn. J Pharm Biomed Anal. 2009;49:55–63.
17. Ming Q, Shuo Y, Hong W, Majumdar P, Leigh N and Glaser R. Ammonia eliminationfrom protonated nucleobases and related synthetic substrates. J Am Soc Mass Spectrom. 2007;18:2040–2057.
18. Calza P, Medana C, Francesco V, Giancotti C and Baiocchi. Characterization of intermediate compounds formed upon photo induced degradationof quinolones by high-performance liquid chromatography/high-resolution multiple-stage mass spectrometry, Rapid Commun Mass spectrom. 2008;22:1533–1552.
19. Rice JM and Dudek GO. Mass spectra of nucleic acid derivatives. II. Guanine,adenine, and related compounds. J Am Chem Soc. 1967;89:2719–2726.