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Development and validation of an LC–UV method for the quantification and purity determination of the novel anticancer agent C1311 and its pharmaceutical dosage form

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Abstract

C1311 (5-[[2-(diethylamino)ethyl]amino]-8-hydroxyimidazo [4,5,1-de]-acridin-6-one-dihydrochloride trihydrate) is the lead compound from the group of imidazoacridinones, a novel group of rationally designed anticancer agents. The pharmaceutical development of C1311 necessitated the availability of an assay for the quantification and purity determination of C1311 active pharmaceutical ingredient (API) and its pharmaceutical dosage form. A reversed-phase liquid chromatographic method (RP-LC) with ultraviolet (UV) detection was developed, consisting of separation on a C18 column with phosphate buffer (60 mM; pH 3 with 1 M citric acid)–acetonitrile–triethylamine (83:17:0.05, v/v/v) as the mobile phase and UV-detection at 280 nm. The method was found to be linear over a concentration range of 2.50–100 μg/mL, precise and accurate. Accelerated stress testing showed degradation products, which were well separated from the parent compound, confirming its stability-indicating capacity. Moreover, the use of LC–MS and on-line photo diode array detection enabled us to propose structures for four degradation products. Two of these products were also found as impurities in the API and more abundantly in an impure lot of API.

Keywords: C1311; Reversed-phase liquid chromatography; Degradation products; Mass spectrometry detection; Photo diode array detection

1. Introduction

C1311 (5-[[2-(diethylamino)ethyl]amino]-8-hydroxyimidazo [4,5,1-de]-acridin-6-one-dihydrochloride trihydrate) is the lead compound from the group of imidazoacridinones, a novel group of rationally designed anticancer agents [1] (Fig. 1). The structure of the imidazoacridinones resembles the anthracyclines (e.g. doxorubicin), the anthracenediones (e.g. mitoxantrone) and the anthrapyrazoles (e.g. oxantrazole and biantrazole), which are known to intercalate DNA and inhibit DNA topoisomerase II. The major structural features of the imidazoacridinones are a planar, polycyclic nucleus (capable of DNA intercalation) and a polyethylenediamine side chain. The addition of an imidazole ring to the acridinone backbone increases the electron density of the π system, making the molecule more resistant to enzymatic reduction into oxygen free radicals, which are known to lead to the dose-limiting cardiotoxicity of the anthracyclines [2]. The hydroxyl group in position 8 increases DNA affinity compared to unsubstituted- or alkyl-substituted derivatives [3,4]. The proposed mechanism of action of C1311 is partly topoisomerase II inhibition, induction of lysosomal rupture and apoptosis in tumor cells without irreversible inhibition of DNA synthesis [5–10]. C1311 exhibited significant cytotoxic activity in vitro toward several tumors and both in vitro and

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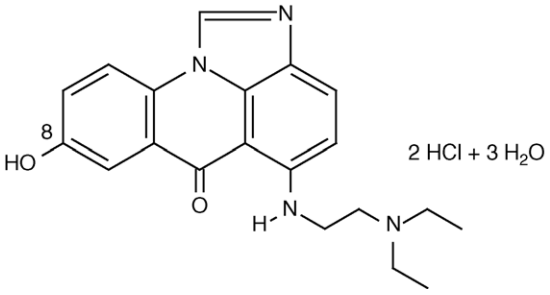

Fig. 1. Chemical structure of C1311 ($C_{20}H_{22}N_4O_2 \times 2HCl \times 3H_2O$, $M_w = 477$).

in vivo toward a range of colon tumors (both murine and human) [5–7,11,12]. The pharmaceutical development of a parenteral dosage form for C1311 necessitated the availability of an assay [13,14] for the quantification and purity determination of C1311 active pharmaceutical ingredient (API) and its pharmaceutical dosage form. The development of a reversed-phase liquid chromatography (RP-LC) method with ultraviolet detection (UV) for this purpose is described in this paper. Validation of the analytical method was performed according to international guidelines [15,16]. On-line photo diode array (PDA) detection and LC coupled to mass spectrometry (MS) were used to attain more information on the observed impurities and degradation products of C1311.

2. Materials and methods

2.1. Chemicals

C1311 as its dihydrochloric salt (trihydrate) (M_w 477) was provided by Xanthus Life Sciences, Inc. (Cambridge, MA, USA). C1311 final product was manufactured in-house (Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands) by freeze-drying. Acetonitrile and methanol were purchased from Biosolve Ltd. (Amsterdam, The Netherlands). Triethylamine and water for chromatography were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade and used without further purification.

3. Methods

3.1. Sample preparation

3.1.1. C1311 standard reference solution

C1311 standard reference solution was prepared by accurately weighing approximately 0.68 mg reference standard and subsequent dissolution in 10 mL of mobile phase, to obtain a final concentration of 68 μg/mL C1311, corresponding to 50 μg/mL anhydrous C1311 free base. The standard reference solution was stored at −20 °C until analysis.

3.1.2. C1311 calibration and quality control samples

C1311 stock solution was prepared by accurately weighing 1.36 mg of C1311 API and subsequent dissolution in 10 mL of mobile phase corresponding to 100 μg/mL anhydrous C1311 free base. Calibration samples and quality control samples were prepared from two separately weighed stock solutions. To obtain sample solutions containing 2.5, 5, 10, 25, 50 and 100 μg/mL C1311 (anhydrous free base), subsequently 25, 50, 100, 250, 500, and 1000 μL of stock solution was transferred to an autosampler vial. Mobile phase was added to a final volume of 1000 μL. Samples were stored at −20 °C until analysis.

3.1.3. C1311 API

C1311 drug substance sample was prepared by accurately weighing 0.68 mg of API and subsequent dissolution in 10 mL of mobile phase, corresponding to 50 μg/mL anhydrous C1311 free base. Samples were stored at −20 °C until analysis.

3.1.4. Pharmaceutical dosage form

C1311 (anhydrous free base) 100 mg/vial lyophilised powder for intravenous use was dissolved in 25 mL of mobile phase. One hundred and twenty five microliters of the resulting solution was diluted to 10 mL with mobile phase. The resulting solution, corresponding to a C1311 concentration of 50 μg/mL (anhydrous free base), was transferred to an autosampler vial. Samples were stored at −20 °C until analysis.

3.2. Liquid chromatography

The HPLC system consisted of an 1100 Series binary HPLC pump, Model G1312A (Agilent Technologies, Amstelveen, The Netherlands), a Model SpectraSERIES AS3000 automatic sample injection device, equipped with a 100 μL sample loop (Thermo Separation Products, Breda, The Netherlands), and a photo diode array detector (PDA) Model Waters™ 996 (Waters Chromatography B.V., Etten-Leur, The Netherlands). Chromatograms were processed using Chromeleon software (Dionex Corporation, Sunnyvale, CA, USA). Separation was achieved using a Zorbax SB-C_{18} analytical column (150 mm × 4.6 mm ID, particle size 3.5 μm, Rockland Technologies Inc., Newport, DE, USA), which was protected by a guard column packed with reversed-phase material (3 mm × 10 mm) (Chrompack, Middelburg, The Netherlands). The mobile phase consisted of phosphate buffer (60 mM; pH 3 with 1 M citric acid)–acetonitrile–triethylamine (83:17:0.05, v/v/v). The flow rate was 0.8 mL/min and UV-detection was performed at 280 nm. The injection volume was 10 μL. A run time of 20 min was employed for the standard samples (C1311 standard reference solution, C1311 calibration and quality control samples), and a run time of 60 min to determine any late eluting impurities and degradation products in the API or pharmaceutical dosage form.

3.3. Liquid chromatography/mass spectrometry

The mobile phase used for the LC/MS experiments was composed of formate buffer (pH 3; 60 mM)–acetonitrile–triethylamine (83:17:0.05, v/v/v). The LC system consisted of an HP1100 liquid chromatograph (Agilent Technologies) with a binary pump, autosampler, and degasser. Other LC conditions were as described above. The eluate was led into the interface of an API 2000 triple quadrupole MS equipped with an electrospray ionisation (ESI) source (Sciex, Thornhill, Ont., Canada). The MS was operated in the positive ion mode. The ion spray voltage was 5500 V and the source temperature was set at 450 °C. A range of *m/z* 30–800 was scanned for the identification of unknown degradation products, using a stepsize of 0.10 amu with dwell times of 3 s. The nebulizer gas (compressed air), turbo gas (N_2), curtain gas (N_2), and collision activated dissociation gas (N_2) were operated at 40, 80, 30, and 2 psi, respectively.

3.4. Validation procedure

The LC method was validated with respect to the following parameters: linearity, accuracy, precision, stability-indicating capability, and stability of standard reference solutions [15,16].

3.4.1. Linearity

Calibration curves at six concentration levels (2.5, 5, 10, 25, 50 and 100 μg/mL C1311 (anhydrous free base) in mobile phase) were analysed in duplicate in three separate runs. Least-squares analysis of concentration, weighted by [1/concentration], versus the area of the C1311 peak was applied. The linearity of the calibration curves was evaluated by means of back-calculated values of the calibration standards, the deviation in response factors at different concentration levels and the observed correlation coefficients of the calibration curves [17].

3.4.2. Accuracy and precision

Accuracy, within-run and between-run precisions of the method were determined by assaying quality control samples at three concentration levels (5, 10 and 50 μg/mL C1311 (anhydrous free base) in mobile phase) in triplicate in three separate analytical runs. Accuracy was measured as the percent deviation from the nominal concentration. The within-run and between-run precisions were calculated by analysis of variance (ANOVA) for each test concentration using the analytical run as the grouping variable. From the ANOVA analysis the day mean square (DayMS), error mean square (ErrMS) and grand mean (GM) were obtained. Within-run and between-run precisions were defined using Eqs. (1) and (2), respectively, where *n* is the number of replicates:

$$\text{Within-run precision (\%)} = 100 \times \frac{\sqrt{\text{ErrMS}}}{\text{GM}} \quad (1)$$

$$\text{Between-run precision (\%)} = 100 \times \frac{\sqrt{(\text{DayMS} - \text{ErrMS})/n}}{\text{GM}} \quad (2)$$

3.4.3. Stability-indicating capability

The stability-indicating capability of the LC method was tested by subjecting C1311 stock solutions in the concentration of 10 mg/mL C1311 (anhydrous free base) in water to several stress conditions for 1 h (oxidation) or 24 h (acid, alkaline, and heat) [16]. Heat: 1 mL of C1311 stock solution was exposed to 100 °C. Oxidation: to 1 mL of C1311 stock solution, 1 mL of a 30% hydrogen peroxide solution was added. Acidic: to 1 mL C1311 solution 0.5 mL of 4 M hydrochloric acid (HCl) was added. Samples were neutralised using 4 M sodium hydroxide (NaOH). Alkaline: similar to the method described under "acidic", using 4 M NaOH as alkalizer and 4 M HCl to neutralise the sample solution. All samples were diluted with mobile phase to a theoretical concentration of 50 μg/mL C1311 (anhydrous free base) before analysis.

3.4.4. Stability of standard reference solutions

Standard reference solutions were stored at ambient temperature (20–25 °C) and ambient light (day–night cycle) conditions and C1311 area and purity were monitored in time.

The C1311 content and purity of C1311 standard reference solutions stored at −20 °C (in the dark) was determined after 24 days and compared with freshly prepared standard reference solutions.

4. Results and discussion

4.1. LC–UV method development

The HPLC method with fluorescence detection developed for the analysis of C1311 in mouse and human plasma [18] was taken as a starting point in the development of a stability-indicating method for the quantification and purity determination of C1311 API and its pharmaceutical dosage form. To achieve the sensitivity required for the analysis of C1311 in biological samples, fluorescence detection was employed. For the analysis of C1311 API and pharmaceutical dosage form with relatively high drug concentrations and possible impurities and degradation products present a less specific mode of detection, e.g. UV, was preferred. The absorption spectrum of C1311 showed maxima at approximately 252, 280, and 422 nm. Using a mobile phase composition of phosphate buffer (60 mM; pH 4 with 1 M citric acid)–acetonitrile–triethylamine (80:20:0.07, v/v/v), C1311 eluted as a single peak with a retention time of approximately 20 min. Peak fronting, however, was considerable. Excluding triethylamine resulted in extensive peak tailing, probably due to the ionic interactions with the silanol groups of the stationary phase. For both conditions (with and without triethylamine), peak width at baseline was >3 min. Next, pH,

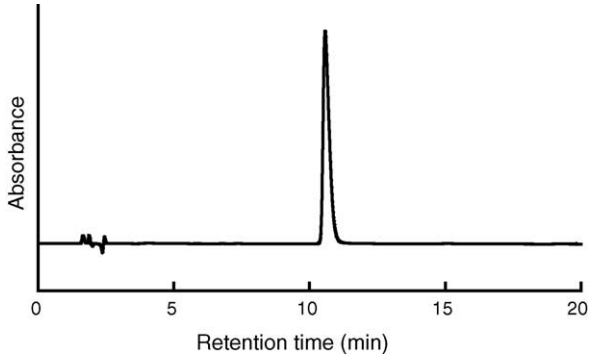

Fig. 2. Representative chromatogram of 50 μg/mL C1311 drug substance solution. The retention time of C1311 is approximately 11 min (k': 6.1). Detection: 280 nm.

buffer concentration, and triethylamine concentration were optimised for C1311 peak shape. The optimised mobile phase consisted of phosphate buffer (60 mM; pH 3 with citric acid 1 M)–acetonitrile–triethylamine (83:17:0.05, v/v/v). This resulted in acceptable tailing factors (<1.8), theoretical plates (>10,000), and peak width at baseline (<1.5 min).

The calibration curves in the range of 10–250 μg/mL C1311 (anhydrous free base) all showed negative intercepts. For C1311, which was shown to adsorb to several materials when solubilised at neutral pH in earlier experiments (data not shown), adsorption of the analyte to the injection device was suspected. Indeed, after injection of the highest calibration standard 2.1% of the C1311 area found was recovered after subsequent injection of mobile phase at pH 3. When, however, the solvent used for the calibration samples (water–acetonitrile 83:17, v/v) was replaced by phosphate buffer (60 mM; pH 3 with 1 M citric acid)–acetonitrile–triethylamine (83:17:0.05, v/v/v) the intercepts became zero.

A representative chromatogram of C1311 (anhydrous free base) 50 μg/mL is given in Fig. 2.

4.2. Validation

4.2.1. Linearity, accuracy and precision

Linearity, accuracy, within-run and between-run precisions of the LC–UV method over the concentration range of 2.50–100 μg/mL was examined. This range corresponds to 5–200% of the intended test concentration of 50 μg/mL for the pharmaceutical quality control of C1311 API and the drug in its pharmaceutical dosage form. The assay was linear with a relative standard deviation of 1.4% in the response factors (area divided by concentration) obtained in the tested concentration range and correlation coefficients > 0.9999 found for all three calibration curves. The average back-calculated concentration for the different calibration concentration levels in the six analytical runs varied between 98.8 and 101.6% of the theoretical concentration for the tested concentration range. The maximal deviation from the nominal concentration was 3.2%, which was found in the lowest calibration level. Within- and between-run precisions were ≤0.9% with accuracies between 98.6 and 99.4% (Table 1).

Table 1
Results of C1311 quality control samples (n = 3 per concentration level)

Concentration (nominal, μg/mL)	Within-run precision (%)	Between-run precision (%)	Accuracy (%)
5.05	0.9	0.2	99.4
10.10	0.3	0.2	98.6
50.51	0.3	n.a.	99.0

n.a.: no significant additional variation due to the performance of the assay in different runs.

4.2.2. Stability of the standard reference solution

The C1311 area of the standard reference solutions was constant during at least 34 h when stored at ambient light (day–night cycle) and temperature (20–25 °C). C1311 purity, however, declined after 8 h of storage, with increasing peak area percentages at relative retention times of 0.60 and 1.14 corresponding to products 4 and 8 observed during accelerated stress testing (see Section 4.2.3).

The C1311 content of standard reference solutions (n = 2) stored at −20 °C (dark) was stable for at least 24 days (98.8 ± 0.7% of the initial content) with no significant increase in degradation products.

4.2.3. Stability-indicating capability

The stability-indicating capability of the assay was examined by accelerated stress testing. Table 2 lists the relative peak areas of C1311 and additional impurities or degradation products observed during accelerated stress testing. Additional products were only included in the table when present at relative peak areas of at least 0.1% in one of the analysed samples. For the accelerated stress testing at elevated temperature, only additional products with relative peak areas exceeding 0.6% are given.

Only minor degradation was observed after subjecting C1311 stock solution to oxidation with hydrogen peroxide for 1 h, and to acidic and alkaline conditions for 24 h, with all degradation peaks well separated from the parent peak. After exposure to heat for 24 h, extensive decomposition was observed. Several degradation peaks evolved and some existing impurities increased. C1311 peak area decreased to 40% of the initial value after 24 h of heat (100 °C) exposure. All degradation peaks were well separated from the parent peak (product 7 in Fig. 3A).

In the chromatogram of C1311 API lot C1-090204 in mobile phase, products 3, 4, 6 and 8–12 were visible in addition to C1311 (product 7) (Table 2). Products 3, 4, 8 and 10–12 were more abundantly present after exposure to one or more of the stress conditions, indicating that these products are degradation products of C1311. The chromatogram of C1311 API lot C1-100703, which showed a relatively high content in products 4 and 8–12 in comparison to API lot C1-090204 resulted in the rejection of this lot of API for manufacture (Table 2).

Table 2
Relative peak areas (%) of C1311 (compound 7), impurities and degradation products in C1311 API and after exposure of API C1-090204 in solution to oxidation, heat, acidic, and alkaline conditions compared to C1311 reference standard

Compound	Rrt	Reference standard	API lot		Acidic	Alkaline	Oxidation	Heat
			C1-090204	C1-100703				
1	0.26–0.29	–	–	–	0.11	–	–	0.67
2	0.30–0.32	–	–	–	0.04	0.41	0.11	10.34
3	0.36–0.38	0.06	0.51	0.58	–	0.09	0.25	31.31
4	0.58–0.60	–	0.04	1.22	–	–	–	8.92
5	0.61–0.62	–	–	–	–	–	–	0.77
6	0.69	0.06	0.10	0.06	0.10	0.12	0.11	0.08
7	1.0 (C1311)	99.72	98.61	95.06	98.98	98.46	98.37	40.55
8	1.13–1.16	0.16	0.10	0.59	0.18	0.09	0.19	1.11
9	1.55	–	0.09	0.59	–	–	–	–
10	1.79–1.87	–	0.05	0.19	–	–	–	2.64
11	3.36–3.49	–	0.24	0.99	0.24	0.36	0.35	–
12	4.57–4.74	–	0.06	0.73	0.12	0.22	0.26	–

Rrt: relative retention time.

The UV spectra of the C1311 peak recorded during the forced degradation studies at the different conditions were identical to the initial spectrum. LC–MS analysis also showed no additional ions. This indicates that there were no degradation products co-eluting with C1311.

To elucidate some of the structures of the degradation products observed during the forced degradation studies, the LC system was coupled to MS. For MS detection, buffer components present in the mobile phase are preferably volatile in order to prevent signal suppression and pollution of the MS interface and quadrupoles. The mobile phase used for the HPLC–UV analysis containing 60 mM of phosphate buffer was therefore not suitable for introducing the analytes into the MS interface. To render a mobile phase compatible with MS detection and with comparable chromatographic properties, 60 mM volatile formate buffer instead of phosphate–citrate buffer was tested with HPLC–UV. The chromatogram obtained after injection of C1311 standard reference solution was similar to the chromatogram obtained with phosphate–citrate buffer with respect to retention and peak shape. Fig. 3 depicts the chromatograms of a degraded C1311 solution (24 h of heat (100 °C) exposure) obtained with phosphate–citrate buffer (A) and formate buffer (B) in the mobile phase. Degradation peaks were matched using the UV spectra obtained with on-line PDA detection. A solution of 1 μg/mL of C1311 in 80% (v/v) methanol was continuously infused (5 μL/min) into the ESI source and mass spectra were recorded in the first quadrupole (Q1) of the MS (Fig. 4). C1311 contains secondary and tertiary amine moieties, suggesting uncomplicated positive ionisation in the electrospray source. Indeed, the $[M+H]^+$ ion of C1311 anhydrous base (*m*/*z* 351) was most abundantly present in the spectrum and only a small peak of the sodium adduct was observed. MS/MS analysis was performed by selecting the *m*/*z* value for the molecular ion and inducing it to fragment in the collision

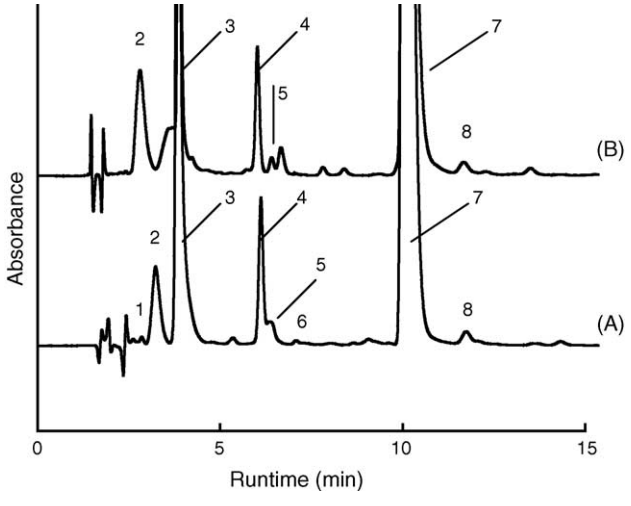

Fig. 3. Degradation chromatogram of C1311 exposed to heat (100 °C) for 24 h using (A) phosphate buffer (60 mM; pH 3 with citric acid 1 M)–acetonitrile–triethylamine (87:13:0.05, v/v/v) or (B) formate buffer (60 mM; pH3)–acetonitrile–triethylamine (87:13:0.05, v/v/v) as the mobile phase with detection at 280 nm. The numbers correspond to the compounds given in Tables 2 and 3.

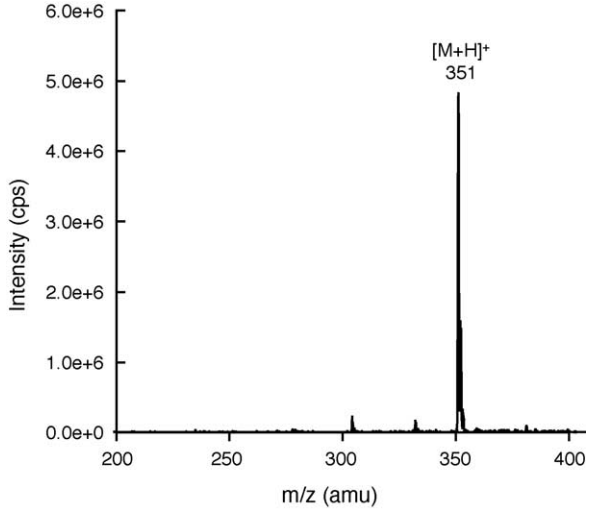

Fig. 4. Positive Q1 spectrum of C1311 using continuous infusion.

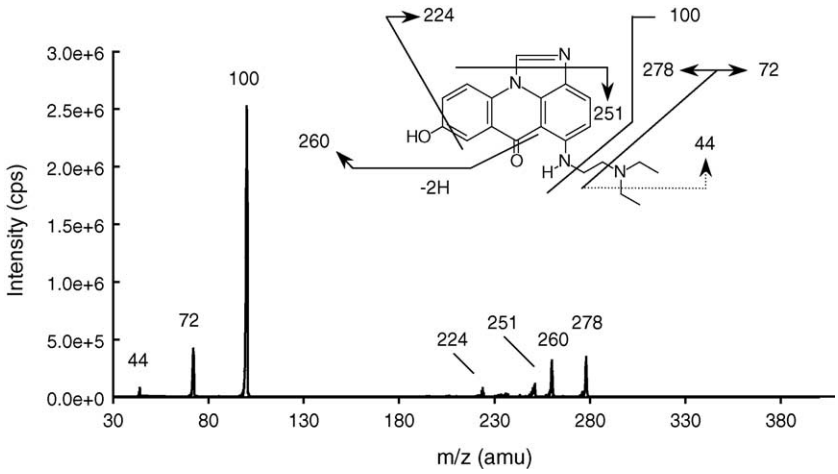

Fig. 5. Positive MS/MS spectrum of C1311. The molecular ion (*m*/*z* 351) was selected for MS/MS analysis.

Table 3
MS and UV data of C1311 (product 7) and degradation products 2–5 obtained after exposure to heat

Product	*m*/*z* values MS $[M+H]^+$	*m*/*z* values MS/MS $[M+H]^+$	UV maxima (nm)
2	367	338; 309; 294; 281; 266	261; 276; 333; 432
3	339	310; 295; 281; 267; 254	261; 282; 343; 434
4	340	311; 296; 282; 267	259; 282; 348; 443
5	349	320; 305; 291; 277	255; 280; 365; 415
7	351	278; 260; 251; 224; 100; 72; 44	251; 282; 379; 422

cell using nitrogen gas, after which the fragments were detected in the third quadrupole of the MS. In the product ion mass spectrum of C1311 (Fig. 5) the most intense peak at *m*/*z* 100 is indicative of cleavage of the diethylaminoethylamino side chain from the molecule. This fragmentation reaction also occurred in Q1 of the MS when the declustering potential was increased from 26 to 81 V, demonstrating the ease of this reaction and offering the potential to perform MS/MS for this particular fragment. The product ion *m*/*z* 100 generated fragments *m*/*z* 72 and *m*/*z* 44, confirming that *m*/*z* 100 corresponds to the diethylaminoethylamino side chain. The subsequent elimination of carbon monoxide from the phenol moiety resulted in fragment *m*/*z* 224. Partial elimination of the diethylaminoethylamino side chain resulted in fragment *m*/*z* 278 and subsequent water elimination in fragment *m*/*z* 260. Partial elimination of the diethylaminoethylamino chain in combination with elimination of the fragment NCH from the imidazole ring with hydrogen re-arrangement may have resulted in fragment *m*/*z* 251.

For the degradation products susceptible to positive ionisation, Table 3 lists the most abundant *m*/*z* values recorded in the first quadrupole and their corresponding MS/MS fragments observed in the third quadrupole. No *m*/*z* values corresponding to the formation of dimers were observed. The most striking difference between the fragmentation pattern of C1311 and the degradation products was the absence of fragment *m*/*z* 100 in the product ion scan of the degradation products, corresponding to cleavage of the diethylaminoethylamino side chain from the molecule. The loss of neutral fragments with masses of 58 and 44 did indicate partial elimination of the polyethylenediamine side chain. The even *m*/*z* value of 340 observed for degradation product 4 indicates the presence of an odd number of nitrogen atoms in the molecule. This to the contrary of degradation products 2, 3, and 5, with even molecular masses indicating an even number of nitrogen atoms in the molecule, like C1311.

The UV spectra of the different degradation products obtained with on-line PDA detection are given in Fig. 6. The UV spectrum of degradation product 5 was found quite similar

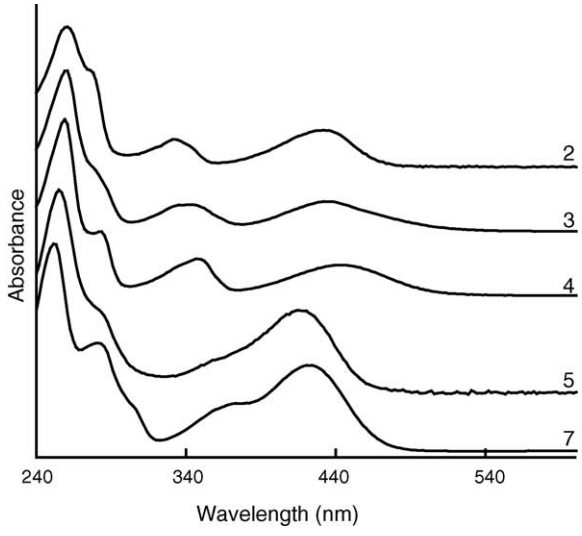

Fig. 6. UV spectra of the products 2–5 and 7 (C1311). The numbers correspond to the compounds given in Tables 2 and 3, and Fig. 3.

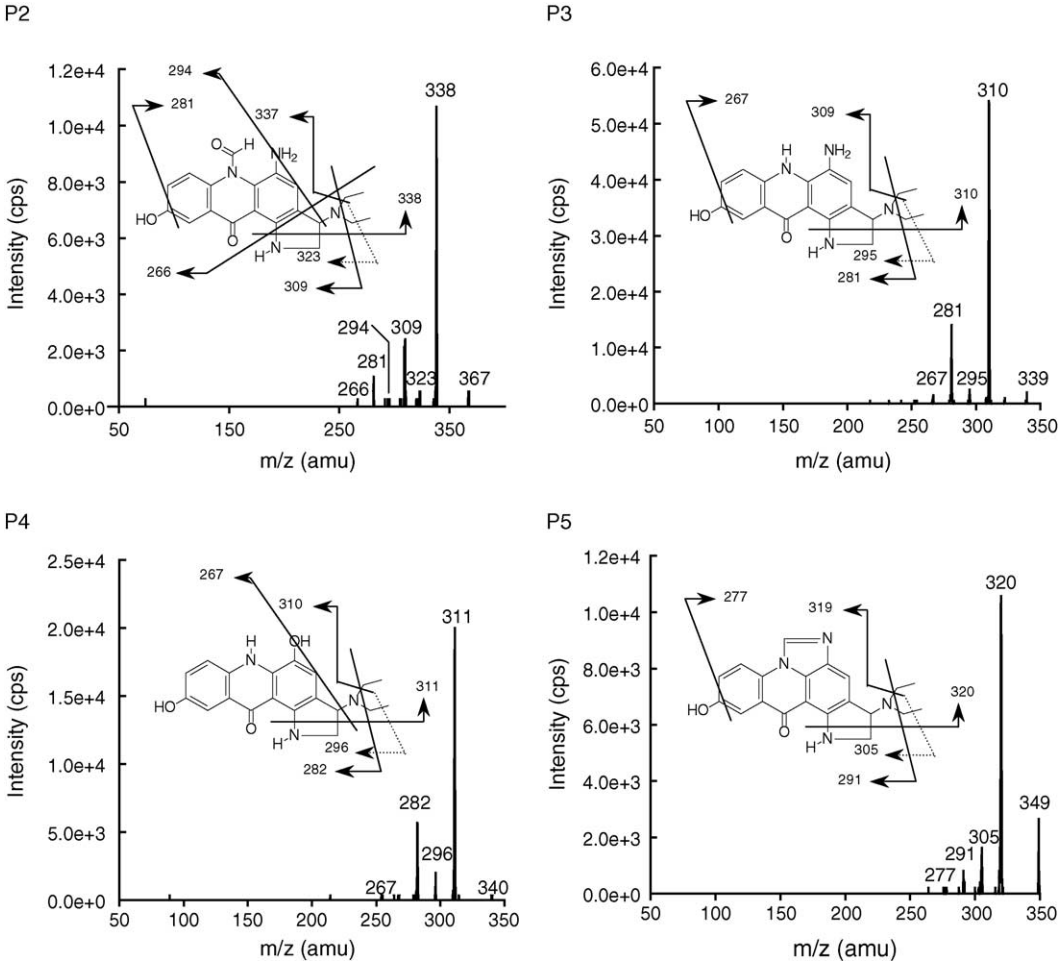

Fig. 7. Positive MS/MS spectrum of the products 2–5 with their proposed structures. The numbers correspond to the compounds given in Tables 2 and 3, and Figs. 3 and 6.

to the spectrum of C1311, suggesting preservation of the imidazoacridinone ring system. The appearance of the spectra of degradation products 2–4, however, clearly deviated from the C1311 spectrum, indicating a modification of the electron density of the heterocyclic ring system.

The proposed structures of the degradation products 2–5 are given in Fig. 7 together with their MS/MS spectra. For all degradation products, cyclisation of the diethylaminoethylamino side chain is proposed, which could explain the observed resistance to cleavage at this site. A similar degradation was earlier seen for mitoxantrone, which showed cyclisation of the alkylamino side chain upon degradation [19].

The proposed degradation products differ from the structures observed after peroxidase-mediated enzymatic oxidation, in which complete dealkylation or deethylation of the diethylaminoethylamino side chain and dimer-like products were observed [20].

The presence of degradation product 3 and 4 as impurities in C1311 API lot C1-090204 was confirmed with LC–MS, as well as the more abundant presence of these degradation products (especially product 4) in the impure C1311 API lot C1-100703.

4.2.4. System suitability parameters

The observed linearity of the analytical method supports the use of a single standard reference concentration for the standardised quantitative analysis of C1311. The system suitability will consist of one blank injection (mobile phase), six injections of the C1311 standard reference solution and one injection of a separately weighed C1311 standard reference solution. Criteria for retention factor, theoretical plates, tailing factor, %rsd in area (six injections), and ratio between the area of the separately weighed standard reference solutions were defined as: $5.5 \leq k' \leq 6.7$, $N > 3000$, $0.8 \leq T \leq 2.0$, %rsd < 1.0%, ratio 0.98–1.02, respectively.

The C1311 content of sample solutions will be calculated using the bracket mode, in which the areas of two preceding and two following standard reference solutions are used.

5. Conclusion

In conclusion, a stability-indicating analytical method comprising of separation with RP-LC and subsequent UV-detection was developed for the novel investigational

anticancer agent C1311. The method was found linear in the range of 2.5–100 μg/mL, precise and accurate. Accelerated stress testing showed degradation products well separated from the parent compound, confirming its stability-indicating capability. Moreover, the use of LC–MS and UV spectra enabled us to propose structures for four degradation products. Two of these products were also found as impurities in the API and more abundantly in an impure lot of API.

The developed stability-indicating LC–UV method will be used for the pharmaceutical quality control of C1311 API and its pharmaceutical dosage form.

References

[1] W.M. Cholody, S. Martelli, J. Paradziej-Lukowicz, J. Konopa, J. Med. Chem. 33 (1990) 49–52.
[2] H.D. Showalter, D.W. Fry, W.R. Leopold, J.W. Lown, J.A. Plambeck, K. Reszka, Anticancer Drug Des. 1 (1986) 73–85.
[3] Z. Mazerska, E. Augustin, A. Skladanowski, M.C. Bibby, J.A. Double, J. Konopa, Drugs Fut. 23 (1998) 702–706.
[4] Z. Mazerska, P. Sowinski, J. Konopa, Biochem. Pharmacol. 66 (2003) 1727–1736.
[5] A.M. Burger, J.A. Double, J. Konopa, M.C. Bibby, Brit. J. Cancer 74 (1996) 1369–1374.
[6] A. Skladanowski, S.Y. Plisov, J. Konopa, A.K. Larsen, Mol. Pharmacol. 49 (1996) 772–780.
[7] E. Augustin, D.N. Wheatley, J. Lamb, J. Konopa, Cancer Chemother. Pharmacol. 38 (1996) 39–44.
[8] J. Mazerski, K. Muchewicz, Acta Biochim. Pol. 47 (2000) 65–78.
[9] N. Zaffaroni, C. De Marco, R. Villa, S. Riboldi, M.G. Daidone, J.A. Double, Eur. J. Cancer 37 (2001) 1953–1962.
[10] J. Dziegielewski, B. Slusarski, A. Konitz, A. Skladanowski, J. Konopa, Biochem. Pharmacol. 63 (2002) 1653–1662.
[11] W.M. Cholody, S. Martelli, J. Konopa, J. Med. Chem. 35 (1992) 378–382.
[12] B. Berger, H. Marquardt, J. Westendorf, Cancer Res. 56 (1996) 2094–2104.
[13] ICH Steering Committee, ICH Q3A(R) Impurities in New Drug Substances, European Agency for the Evaluation of Medicinal Products, International Commission on Harmonisation, London (CPMP/ICH/2737/99), 2002. http://www.ich.org.
[14] ICH Steering Committee, ICH Q3B(R) Impurities in New Drug Products, European Agency for the Evaluation of Medicinal Products, International Commission on Harmonisation, London (CPMP/ICH/2738/99), 2003. http://www.ich.org.
[15] ICH Steering Committee, ICH Q2A Text on Validation of Analytical Procedures, European Agency for the Evaluation of Medicinal Products, International Commission on Harmonisation, London (CPMP/ICH/381/95), 1994. http://www.ich.org.
[16] ICH Steering Committee, ICH Q2B Validation of Analytical Procedures: Methodology, European Agency for the Evaluation of Medicinal Products, International Commission on Harmonisation, London (CPMP/ICH/281/95), 1996. http://www.ich.org.
[17] H. Rosing, W.Y. Man, A. Doyle, A. Bult, J.H. Beijnen, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 329–354.
[18] C.R. Calabrese, P.M. Loadman, J. Chromatogr. B Biomed. Sci. Appl. 690 (1997) 275–281.
[19] J.H. Beijnen, A. Bult, W.J. Underberg, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, Academic Press, California, 1988, pp. 221–258.
[20] Z. Mazerska, J. Dziegielewski, J. Konopa, Biochem. Pharmacol. 61 (2001) 685–694.