Analytical Methods

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c7ay00779e

Supercritical fluid (CO_2) chromatography for quantitative determination of selected cancer therapeutic drugs in the prescence of potential impurities in injection formulations

Venkata Narasimha Rao G.,* [a] Ravi B.,[a] Jalandhar D.,[a] Manoj P.[a] and R. Venkata Nadh[b]

In the present study, two cancer therapeutic drugs (docetaxel and bortezomib) were separated from their potential impurities on a chromatographic platform by utilizing CO_2 gas (supercritical state) and quantified. The chromatographic separations were achieved on two short columns BEH-2EP (100 mm × 3 mm, 1.7 μm) and CHIRALPAK AD-3 (100 mm × 4.6 mm, 3 μm) for docetaxel and bortezomib, respectively. The present work describes the role of organic modifiers in the separation of polar compounds by supercritical fluid chromatography. The two new methods were fully validated in accordance with the current ICH (International Council for Harmonization of technical requirements for pharmaceuticals for human use) guidelines. The stability indicating power of the methods was demonstrated from the stress studies conducted on the injection formulations of the two compounds. The methods are precise with % RSD of 0.4, linear with the correlation coefficient of $r^2 \geq 0.999$ and accurate in the range of 50–150% of the target assay concentration. The two methods can be equally employed for the assay determination of docetaxel and bortezomib APIs as well.

Received 23rd March 2017
Accepted 7th April 2017

DOI: 10.1039/c7ay00779e

rsc.li/methods

1 Introduction

Supercritical fluid carbon dioxide has emerged as a versatile solvent for scientists to use in various chemical operations owing to its sustainable (green) properties, relative chemical inertness, greater applications in manufacturing, and abundance,.[1–5] This resulted in supercritical fluid chromatography (SFC) as an alternative and complementary method to the HPLC technique. The potential of SFC using packed columns for analysis in the pharmaceutical industry has been recognized for many years.[7] SFC can offer highly efficient separations in shorter analysis times and at a low-pressure drop without compromising the resolution, plate counts and tailing factors. However, the lack of reliable and sensitive commercial SFC systems has prevented the extensive use of SFC in the industry. With newly available instruments, several SFC methods have been reported for a variety of compounds.[6–9]

The present study is an application of SFC for the assay determination of docetaxel and bortezomib in injection formulations.

Docetaxel is an antineoplastic agent belonging to the taxane compound family and used in the treatment of several cancers (ovarian, prostate, esophageal, *etc.*) by either monotherapy or in combination with several other drugs like gemcitabine, irinotecan, cisplatin and carboplatin.[10–14] The chemical name of docetaxel is (2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-*tert*-butyl ester, 13-ester with 5β-20epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate. Its formulation is available as Taxotere Injection Concentrate[15,16] in the market.

Bortezomib is available in the market as VALCADE® from Millennium Pharmaceuticals, Inc., and Janssen-Cilag and is used for the treatment of patients with multiple myeloma.[17] Multiple myeloma (MM) is an incurable disease with a poor survival, which has not been affected even by high-dose chemotherapy. Bortezomib appears to improve survival and response rates of patients with previously untreated MM in spite of higher risk of peripheral neuropathy.[18] It is one of the novel agents that has improved rate of remission during ASCT (autologous stem cell transplantation) without increasing toxicity.[19,20]

Bortezomib is a modified dipeptidyl boronic acid. The chemical name of bortezomib is [(1*R*)-3-methyl-1-[[(2*S*)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]amino]butyl] boronic acid.[21] The molecular weight is 384.24, and the molecular formula is $C_{19}H_{25}BN_4O_4$. The chemical structures of docetaxel and bortezomib are shown in Fig. 1.

Currently docetaxel is official in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP), and Indian

[a]*Formulations Research and Development Centre, Mylan Laboratories Ltd, Jinnaram, Medak, 502325, India. E-mail: venkata.nr@gmail.com*
[b]*GITAM University, Bengaluru, Karnataka-562163, India*

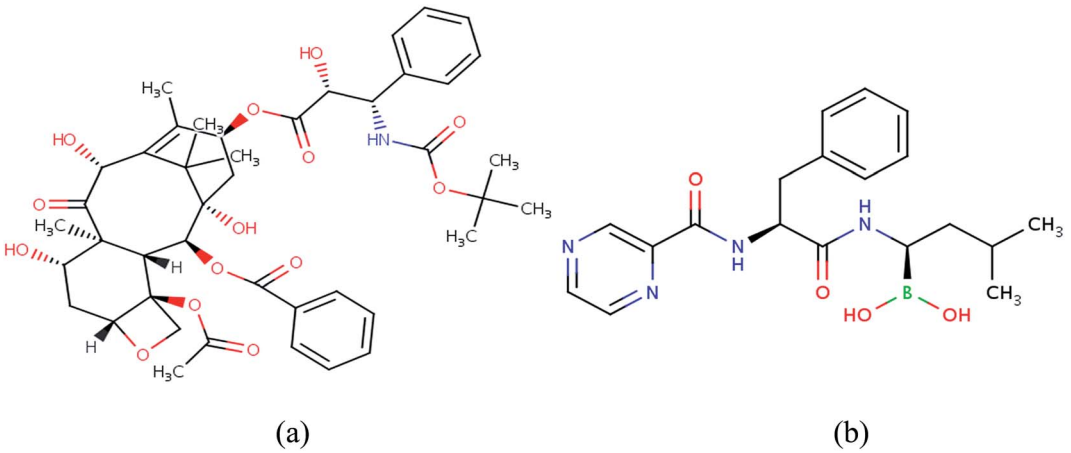

Fig. 1 Chemical structures of (a) docetaxel and (b) bortezomib.

Pharmacopoeia (IP), while bortezomib is not official in any pharmacopoeia.[21–23] Each pharmacopeia has published monographs of docetaxel and docetaxel injection. The USP monograph prescribes a reversed phase, and a 60 minute gradient method for the assay and RS determination using a 150 mm × 4.6 mm, 3.5 μm, L1 column. The mobile phase contains water, acetonitrile and acetic acid mixed in the ratio of 1 : 1 : 0.1, respectively. The detection was carried out at 232 nm, with a sample load of 20 μL. Ph. EP and IP prescribe a common method (72 minutes gradient) for assay and RS, similar to USP. A few HPLC and UPLC coupled with mass spectrometry (HPLC-MS/MS, UPLC-MS/MS) methods for plasma docetaxel are available in the literature.[24–30]

Few analytical methods are available on bortezomib estimation in bulk and formulations.

Chandra Sekhar K *et al.* reported one ultraviolet method for the determination of bortezomib in bulk and dosage form.[31] A few RP-HPLC and HPLC coupled with mass spectrophotometer methods were reported for the estimation of bortezomib and impurities in bulk and injection formulations.[32–40] All these methods use octadecylsilyl columns, and the mobile phase consists of acetonitrile in major proportions, 60%,[35] 65%,[36] 80%,[37] 50% (ref. 38) combined with buffers.

All the above reported methods on docetaxel and bortezomib are reverse phase methods and use a variety of organic solvents in their mobile phases. Until today, there are no reported literature studies available on the utilization of CO_2 as a mobile phase for carrying out chromatographic separations of cancer drugs from their potential impurities. Hence, an attempt is made here and novel methods were developed, which are ecofriendly.

The biggest challenge in developing these methods was the selection of organic modifiers and their quantity in the separation of polar impurities from the main active compound.

2 Materials and methods

2.1 Chemicals and reagents

Isopropyl alcohol was obtained from Rankem (India). HPLC grade methanol and diethylamine (DEA) were procured from Merck, India. Triflouro acetic acid (TFA) was from Acros organics. Ethanol used in the study was obtained from Commercial alcohols. Carbon dioxide gas was purchased from Sai Padmaja Oxygen at Hyderabad, India. The samples of docetaxel injection and bortezomib for injection used in the study were obtained in-house from Mylan (Hyderabad, India). Docetaxel CRS and docetaxel identification CRS were procured from US Pharmacopoeia. Bortezomib standard and its impurities were obtained from an API supplier.

2.2 Instruments and chromatographic conditions

An integrated acquity UPC^2 system was from Waters Corporation, Milford, USA and equipped with a Waters photodiode array detector (PDA). Data collection and analysis were performed using Empower software 2pro (Waters Corporation). Balances used for weighing the reference standards and samples were from Metler and Sartorius. The two columns, namely BEH-2EP and CHIRALPAK AD-3 were procured from Waters India and Diacel respectively.

2.2.1 Chromatographic conditions for docetaxel injection. A BEH-2EP (100 mm × 3.0 mm, I.D. and 1.7 μm) column was used for separating docetaxel and its potential impurities. The column oven temperature was maintained at 45 °C. The mobile phase consisting of liquid CO_2 and a co-solvent (a mixture of ethanol and methanol (1 : 1) containing 0.1% diethylamine) was pumped in an isocratic mode (85 : 15) at a constant flow rate of 1.5 mL min^{-1} throughout the run. 1 μL of the sample was injected and detected at a wavelength of 228 nm. During the analysis, the active backpressure of the system was maintained at 1900 psi. The sample cooler temperature was maintained at 10 °C.

2.2.2 Chromatographic conditions for bortezomib dry powder for injection. For bortezomib the mobile phase consists of liquid CO_2 (% A) and a mixture of 0.5% TFA in an ethanol and IPA mixture (1 : 1) (% B). The mobile phase was pumped into the chromatograph at a flow rate of 1.3 mL min^{-1} using a linear gradient program. The gradient programme at time T (minutes), ramp of % B (organic modifier) is T/% B, 0/10, 1/10, 3/20, 8/20, 8.5/10, 10/10.

Table 1 Results of forced degradation studies[a]

S. no.	Stress condition	Assay of degraded sample	% degradation	Purity angle	Purity threshold	Assay of degraded sample	% degradation	Purity angle	Purity threshold
		Docetaxel				Bortezomib			
1	Acid hydrolysis	78.6	20.5	0.121	0.313	92.2	7.1	1.259	1.870
2	Base hydrolysis	66.9	32.2	0.135	0.456	91.4	7.9	1.647	2.625
3	Thermal degradation	96.6	2.5	0.090	0.323	99.9	0.6	0.985	1.529
4	Oxidation	90.5	8.6	0.106	0.290	81.8	17.5	0.721	1.453
5	Photolytic degradation (UV)	96.7	2.4	0.231	0.421	96.7	2.6	0.978	1.752
6	Photolytic degradation (light)	95.3	3.8	0.100	0.370	93.7	5.6	1.054	1.494

[a] Control sample results for calculating the % degradation, docetaxel assay = 99.1 and bortezomib assay = 99.3.

A CHIRALPAK AD-3 column (100 mm × 4.6 mm I.D., 3 μm particle size) from Diacel was used for the separation of bortezomib and its impurities. The column oven temperature was kept at 45 °C throughout the experiments. A sample volume of 1 μL was injected into the chromatograph and detected at 270 nm. The active backpressure of the system was maintained at 1900 psi throughout the analysis. The sample cooler temperature was maintained at 10 °C.

2.3 Standard and sample preparations

2.3.1 Diluent preparation. HPLC grade methanol was used as the diluent, for the preparation of standards, system suitability solutions, and samples for both the methods. This selection was based on the solubility studies conducted on the two active drug substances.

2.3.2 Docetaxel standard, USP identification solution (system suitability) and sample preparation. An amount of docetaxel USP CRS was weighed accurately in a suitable volumetric flask. 10 mL of the diluent was added, sonicated to

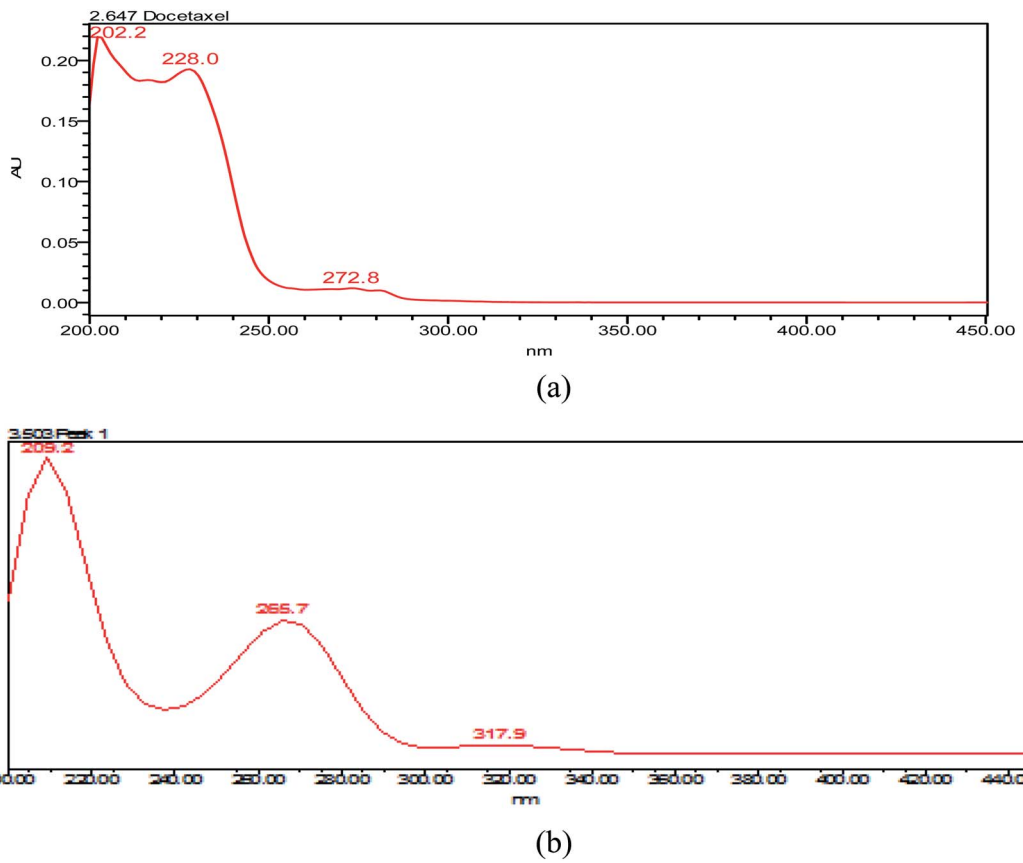

Fig. 2 Ultraviolet-Visible spectra of (a) docetaxel and (b) bortezomib.

dissolve the contents and diluted suitably to obtain a concentration of 1000 μg mL^{-1}. USP docetaxel identification RS contains docetaxel and small amounts of listed impurities 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epi-docetaxel, and 4-*epi*-6-oxodocetaxel. It was used as a system suitability and spiked sample solution. The sample solution was prepared similar to the USP CRS solution by diluting an appropriate volume of the sample with the diluent to get the concentration of 1000 μg mL^{-1}.

2.3.3 Bortezomib standard and sample preparation. The standard solution was prepared by dissolving an amount of bortezomib in the diluent and diluting suitably to obtain a concentration of 180 μg mL^{-1}. The sample solution was prepared similar to that of the standard to get the concentration of 175 μg mL^{-1}. Bortezomib has seven impurities namely, (*S*,*S*)-diastereomers, (*R*,*R*)-diastereomers, *R*,*S* enantiomer, impurity A, impurity B, impurity C and impurity D. The impurities of bortezomib were prepared by dissolving a suitable quantity in the diluent and spiked into the sample preparation.

2.4 Forced degradation study

2.4.1 For docetaxel injection. Forced degradation studies were performed to assess the stability indicating power of the method. Docetaxel injection samples were deliberately subjected to various stress conditions such as acidic (1 mL of 1 N HCl for 12 h at room temperature), basic (1 mL of 1 N NaOH for 12 h room temperature), oxidative (1 mL of 1% H_2O_2 for 30 minutes), humidity (90% RH for 12 hours) UV light (200 watt hours per square meter), and light (1.2 m lux hours). The stressed samples were further neutralized and diluted suitably to get final concentrations of 1000 μg mL^{-1}. All samples were then chromatographed and the peak purity plots were obtained for the docetaxel peak. Peak purity testing determines the peak homogeneity by comparing each spectrum within the peak against the peak apex. The chromatograms of the stressed samples were evaluated for peak purity of docetaxel using Waters Empower Networking Software. The values of the purity angle and purity threshold were used to estimate the spectrum homogeneity and the peak purity. Table 2 shows the

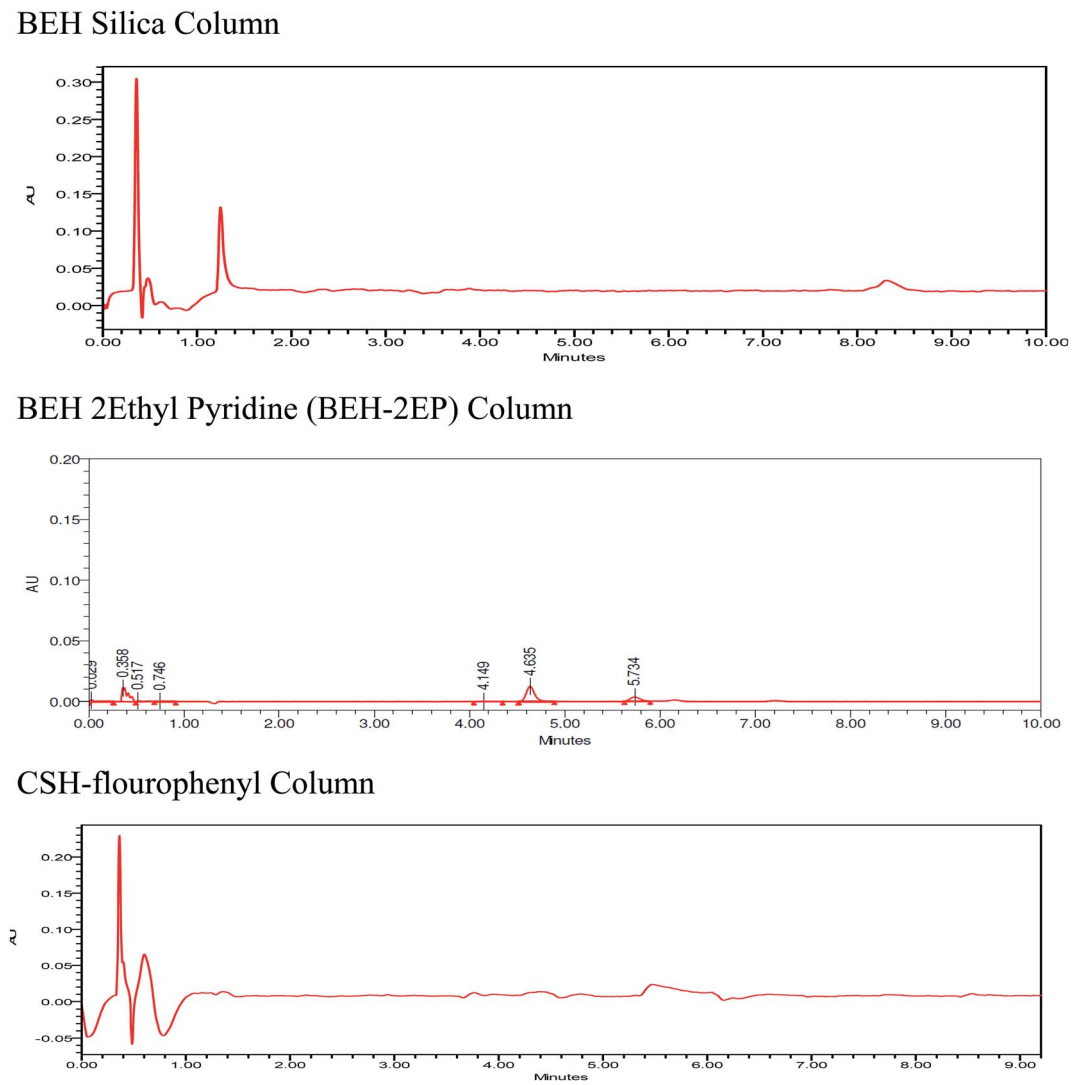

Fig. 3 Chromatograms obtained from primary screenings (Trial-1).

degradation data with purity angle and purity threshold. The criterion for peak homogeneity is that the purity angle should be less than the peak threshold as per the waters empower software. The purity angle and purity threshold from Table 2 show that the docetaxel peak is homogenous in the presence of degradation products as obtained from the stress samples.

2.4.2 For bortezomib injection. Bortezomib for injection samples were subjected to the following stress conditions. Acid hydrolysis (1 mL of 0.5 N HCl for 6 hours), base hydrolysis (1 mL of 1 N NaOH for 4 hours), heat exposure (at 60 °C for 24 hours), oxidative (3 mL of 10% H_2O_2), photolytic exposure (UV-200 watt hours per square meter, light-1.2 million lux hours) studies were carried out as per the current ICH guidelines (international council for harmonisation of technical requirements for pharmaceuticals for human use). The exposed samples were neutralized and diluted to get a concentration of 175 μg mL^{-1}. The bortezomib peak was evaluated for homogeneity by measuring the purity angle and purity threshold. The results are tabulated in Table 1. The purity angle of bortezomib obtained from various stress studies was found less than the purity threshold. This indicates that the bortezomib peak is homogenous in the presence of its degradants.

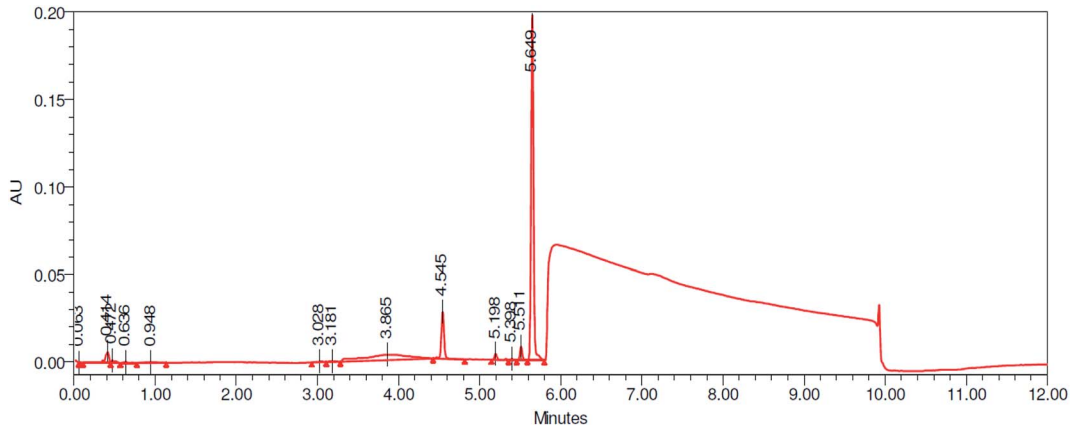

Fig. 4 Chromatograms obtained from Trial 2.

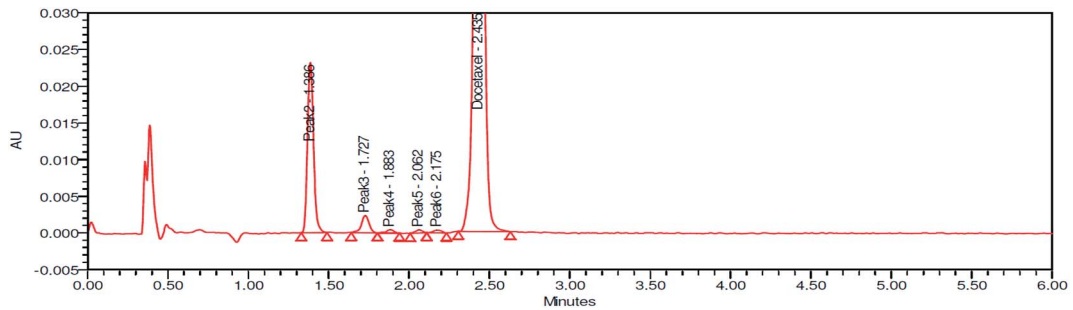

Fig. 5 Development chromatograms obtained from Trial 3.

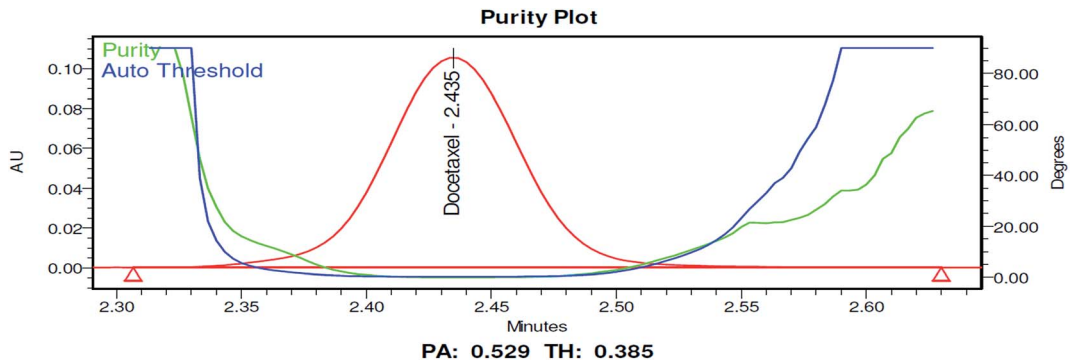

Fig. 6 Purity plot of the docetaxel peak obtained from development Trial 3.

3 Results and discussion

3.1 Method development and optimization

A systematic approach was adopted for developing methods using the SFC principle. The two compounds were studied for ultra-violet absorption to determine the detection lambda maxima. For this, solutions of docetaxel and bortezomib were prepared having appropriate concentrations within the UV-Visible range and absorbance study was conducted. The two UV spectra are shown in Fig. 2. Docetaxel exhibited two maxima, 228 nm, and 272 nm. Bortezomib exhibited three maxima, 209 nm, 266 nm, and 318 nm.

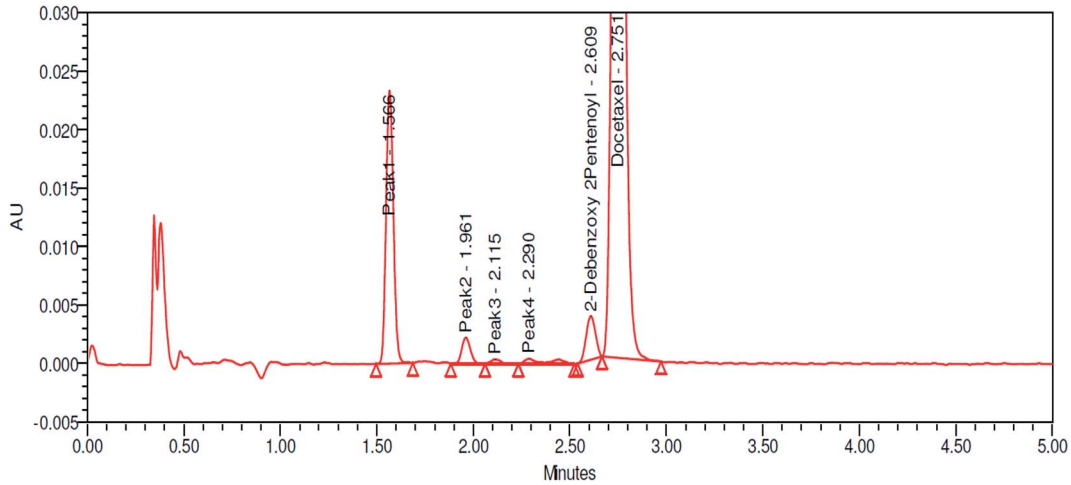

Fig. 7 Typical chromatogram of optimized chromatographic conditions.

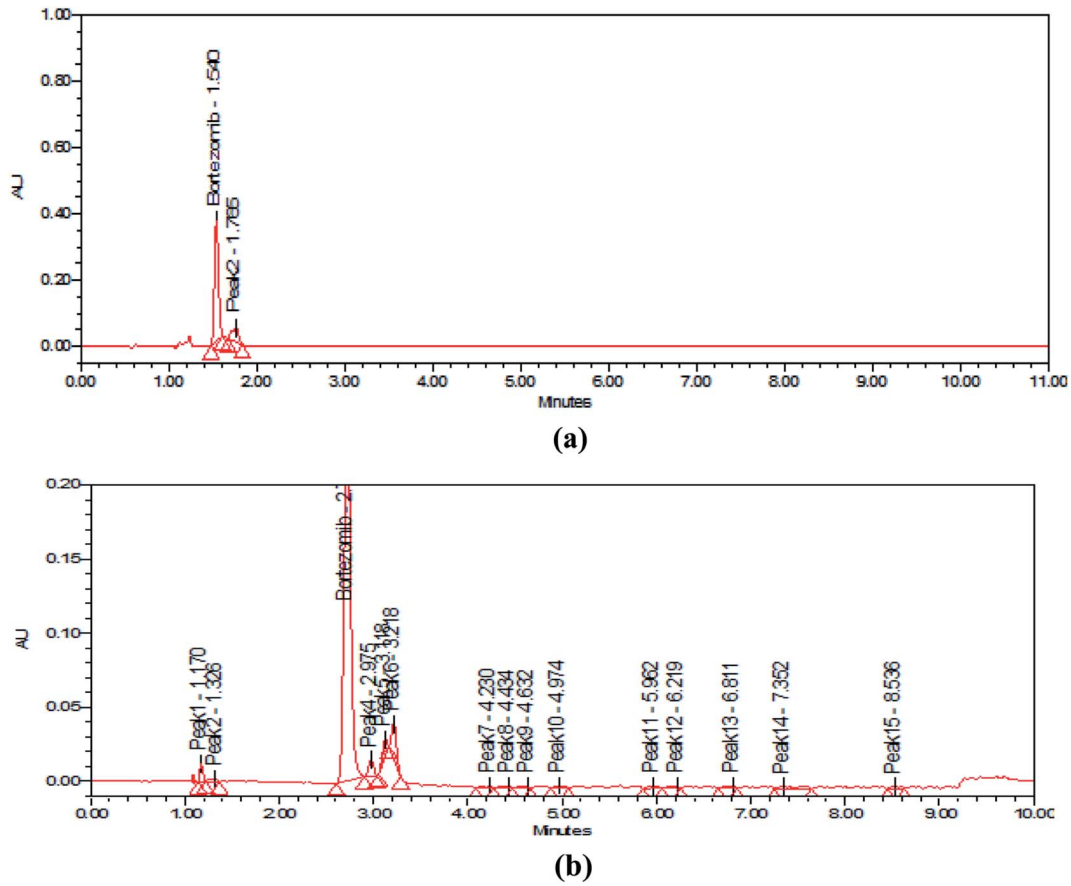

Fig. 8 Chromatograms obtained from primary screenings on (a) BEH-2EP and (b) CHIRALPAK AD-3 (Trial-1).

3.1.1 Determination of docetaxel in docetaxel injection

3.1.1.1 SFC primary screening and optimization of docetaxel

SFC primary screening. ***Trial-1.*** In primary screening three different stationary phases, namely BEH (ethylene bridged hybrid) silica, BEH-2EP (ethylene pyridine), and CSH (charged surface hybrid)-flourophenyl with sub-2 μm particles which are specifically designed for SFC, were employed to have predictable separations of sample components. This selection of columns was based on their chemistries, polarities, pressure tolerability (up to 18 000 psi or 241 bar), operable temperature (up to 60 °C) and compatibility with acidic and basic additives like TFA, DEA, *etc.*

In view of the study objective, primary screenings were done with 100% CO_2 mobile phase with a flow rate of 10 mL min^{-1}. The system pressure was maintained at 1900 units through an active back pressure regulator. The initial column temperature was kept at 40 °C. With these set of chromatographic conditions, the first trial was made by injecting USP identification

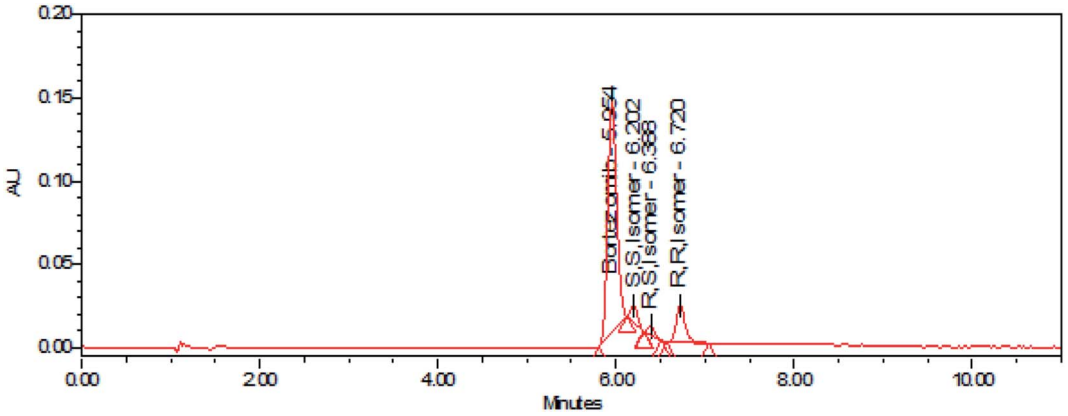

Fig. 9 Development chromatogram obtained from Trial 2.

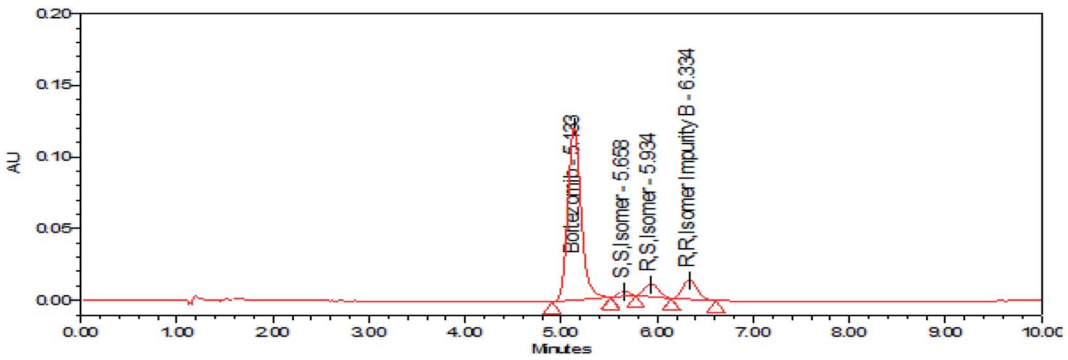

Fig. 10 Development chromatogram obtained from Trial 3.

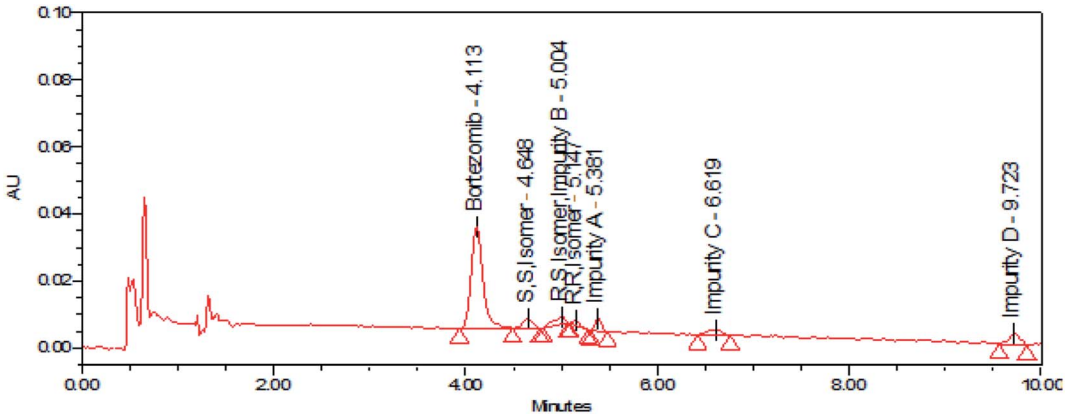

Fig. 11 Development chromatogram obtained from Trial 4.

solution (1 μL) into the chromatographic system. The chromatograms are shown in Fig. 3.

The inference from above chromatograms was that no detectable peaks were observed in BEH silica and CSH flourophenyl columns. BEH-2EP showed some promising retention of peaks with very low response. Hence, further development strategy was continued with the BEH-2EP column. Supercritical CO_2 is a nonpolar solvent with poor solvating power. This is one of the reasons for poor retention of polar compounds. Berger and Deye *et al.*, suggest that the addition of modifiers to the mobile phase can increase the extraction efficiency of CO_2.[41–43]

Trial-2. To increase the mobile phase strength, a few experiments were conducted with a small quantity of ethanol (0–5%)

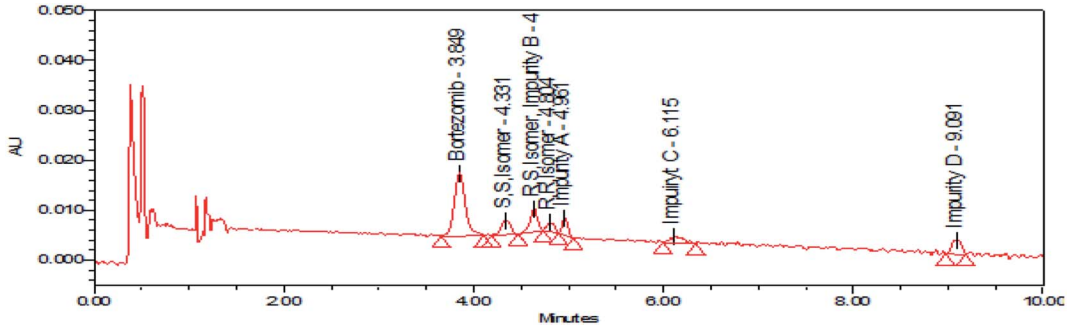

Fig. 12 Typical chromatogram of optimized chromatographic conditions.

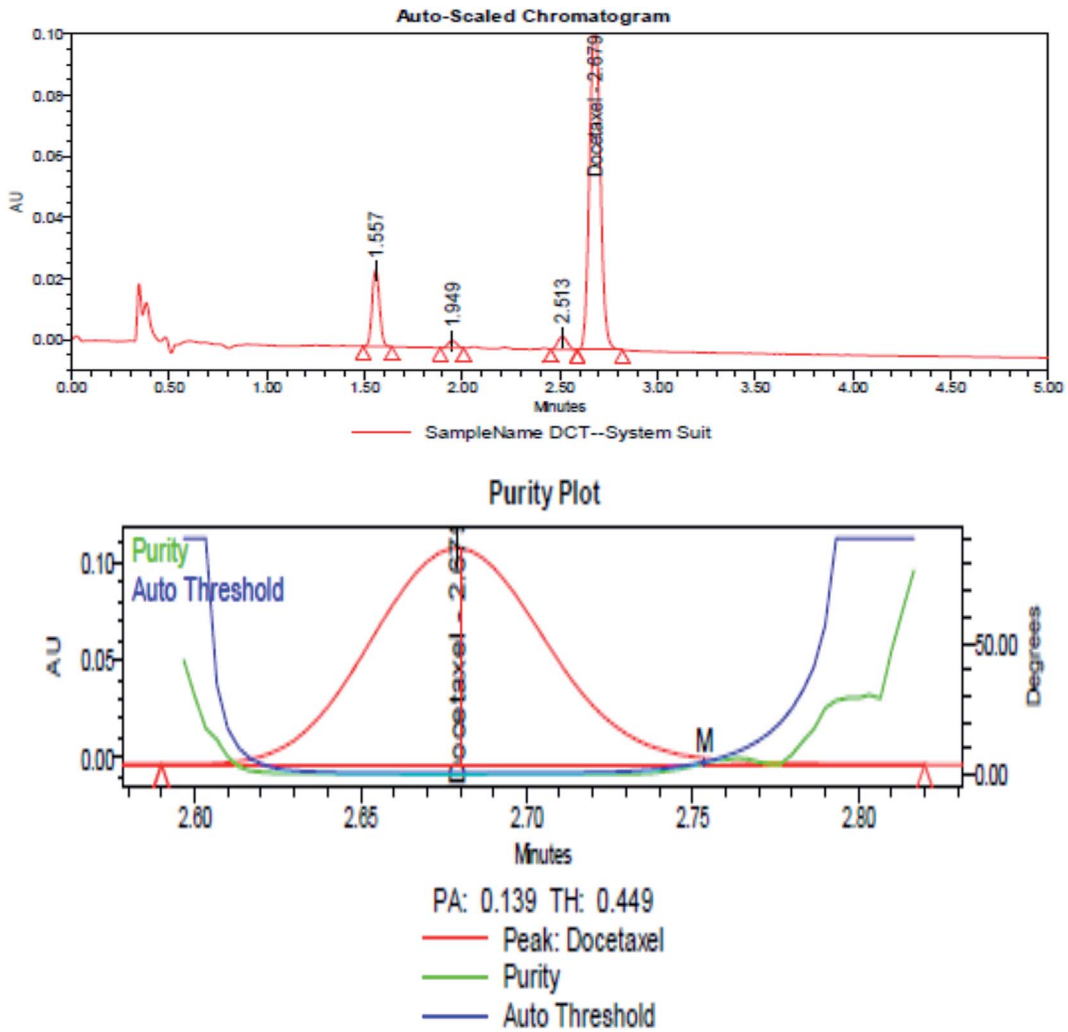

Fig. 13 System suitability chromatogram obtained from docetaxel identification solution.

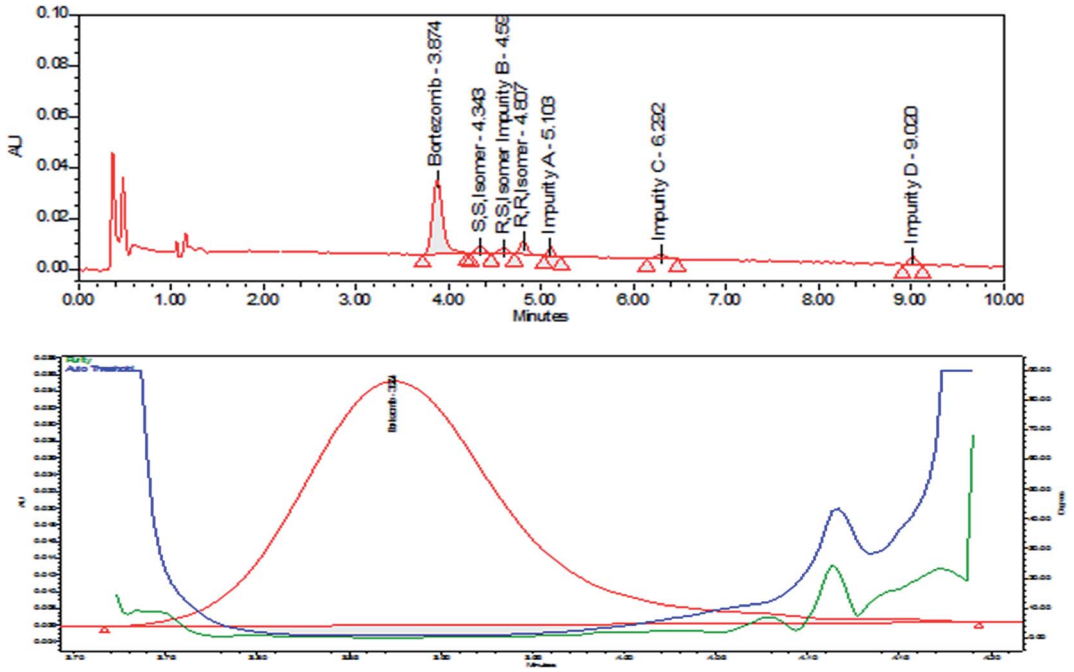

Fig. 14 System suitability chromatogram obtained for bortezomib.

along with a gradient programme. The increase in the polarity of the mobile phase has increased the sample partition and 5% ethanol has resulted in the elution of docetaxel at a RT of about 5.6 minutes. All the other components eluted closely before the docetaxel peak. The chromatogram is shown in Fig. 4.

Trial 3. The separation and elution of majority of components occurred at the isocratic portion (95 : 5 CO_2 : ethanol) of the gradient programme and hence the isocratic composition was finalized to even avoid the gradient hump. With the new chromatographic conditions, the identification solution was injected and the chromatogram was recorded. This trial resulted in a better baseline and improved separations among the components. The specimen chromatogram is shown in Fig. 5.

The corresponding purity plot of the DCTX peak is shown in Fig. 6. The purity angle (PA = 0.529) and threshold (TH = 0.385) indicate that the DCTX peak is not homogenous (criteria: PA < TH). Hence further separation is required.

Trial 4. From the literature, the addition of small amounts, typically 0.1–1% of additives, to the mobile phase has been shown to have a beneficial effect on the peak shape and sensitivity in SFC. Based on this 0.1% of DEA was added to the organic composition. This has further improved the peak shape and resolution of closely eluting peaks. The final optimized chromatogram is shown in Fig. 7.

3.1.1.2 SFC primary screening and optimization for bortezomib

SFC primary screenings. The structural evaluation shows that bortezomib possesses chiral centers and may give rise to chiral related substances. Having this information, the entire method development was strategized combining both chiral and achiral screening and optimization.

CHROMATOGRAMS OBTAINED FROM FORCED DEGRADATION STUDIES

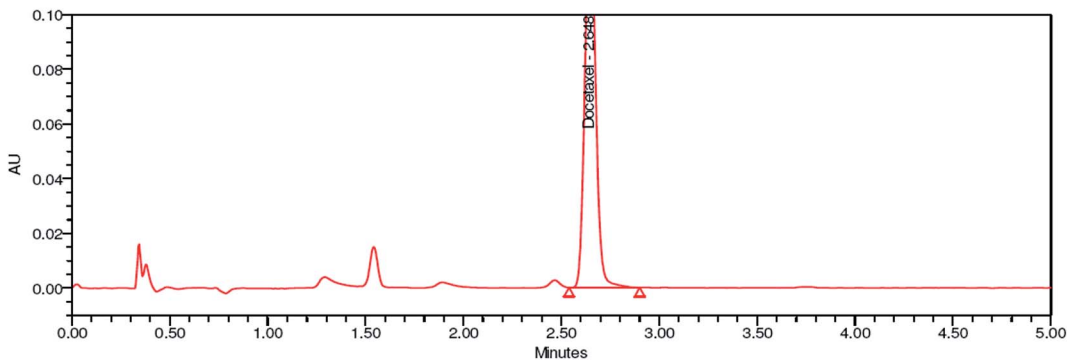

Fig. 15 Acid stressed sample chromatogram.

Trial-1. In primary screening, four columns having different stationary phases namely HSS T3, BEH-2EP (ethylene pyridine), and CSH (charged surface hybrid)-flourophenyl with sub-2 μm particles which are specifically designed for SFC, and amylose tris (3,5-dimethylphenylcarbamate) were employed to have predictable separations of sample components. This selection of columns was based on their chemistries, polarities, pressure tolerability (up to 18 000 psi or 241 bar), operable temperature (up to 60 °C) and compatibility with acidic and basic additives like TFA, DEA, *etc.* The mobile phase was kept 100% CO_2 in view of study objective at a flow rate of 1.0 mL min^{-1}. The system pressure was maintained at 1900 psi through the active backpressure regulator. The initial column to temperature was kept at 45 °C to maintain the supercritical state of CO_2. With these set of chromatographic conditions the bortezomib impurity mixture (1 μL) was injected into the chromatographic system in each of the stationary phase. No peaks were detected on the CSH, and HSS T3 column. BEH-2EP and CHIRAL-AD 3 columns showed few promising peaks. Fig. 8 shows the chromatogram obtained on the two columns, respectively.

Trial-2. Further development strategy was continued with CHIRALPAK-AD 3 (100 × 4.6, 3 μm). According to Berger and Deye *et al.*, addition of organic modifier to the mobile phase increases the extraction efficiency of CO_2. Based on this, ethanol

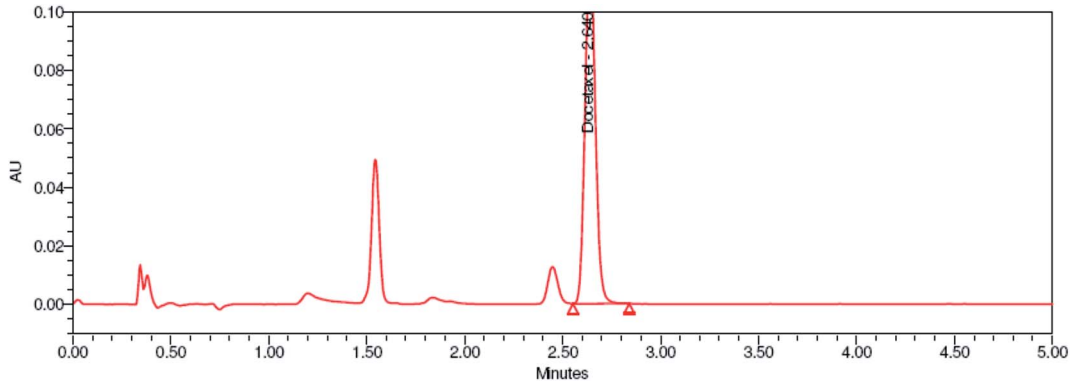

Fig. 16 Base stressed sample chromatogram.

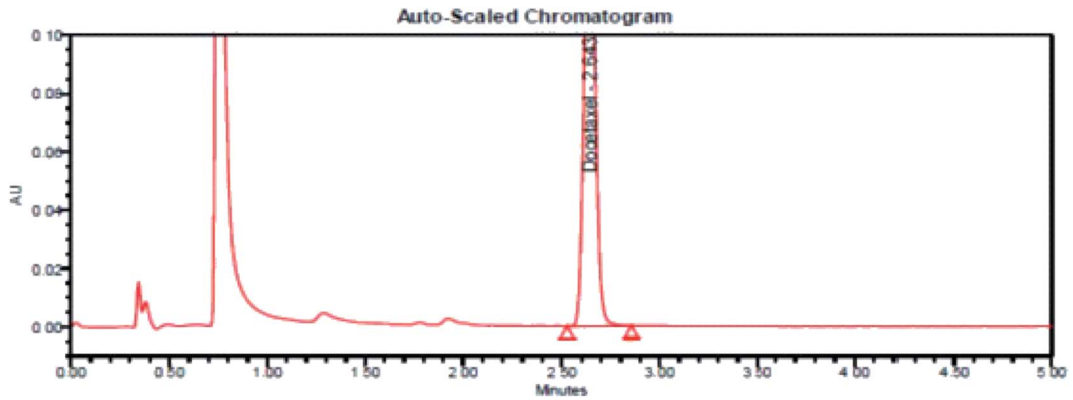

Fig. 17 Peroxide stressed sample chromatogram.

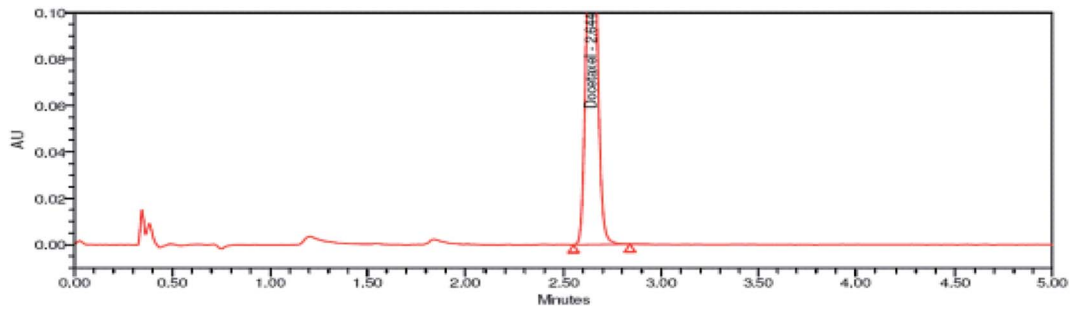

Fig. 18 Thermal stressed sample chromatogram.

was introduced into the mobile phase, and several experiments were conducted with varied percentages using a gradient programme. The increase in polarity of the mobile phase has increased the sample partition. The column temperature and flow rate were reduced to 45 °C and 1.0 mL min^{-1} respectively to check their impact on the separation. With these changes, bortezomib standard solution and individual impurities were injected into the chromatograph. Due to these changes, bortezomib was slightly retained and separation among the four peaks was improved a little. The other three peaks were identified as isomers of bortezomib. The chromatogram obtained from Trial 2 is shown in Fig. 9.

Trial 3 and 4. In Trial 3 IPA was introduced along with ethanol in a 1 : 1 ratio. The gradient programme was slightly modified to improve separation among the closely eluting isomers and elute late-eluting impurities.

This trial resulted in better separation among the isomer peaks, and the other peaks were not eluted within 10 minutes. The obtained chromatogram with Trial 3 is shown in Fig. 10.

In Trial 4 the flow rate was increased from 1.0 to 1.2 mL min^{-1} to check for the other two impurities (impurity C and impurity D). Both impurities were eluted within 10 minutes with good separation. All peaks were eluted and are well

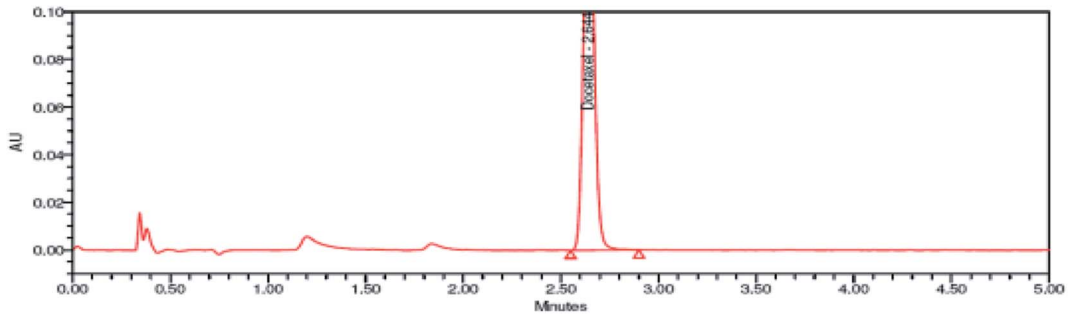

Fig. 19 Sample chromatogram UV degradation.

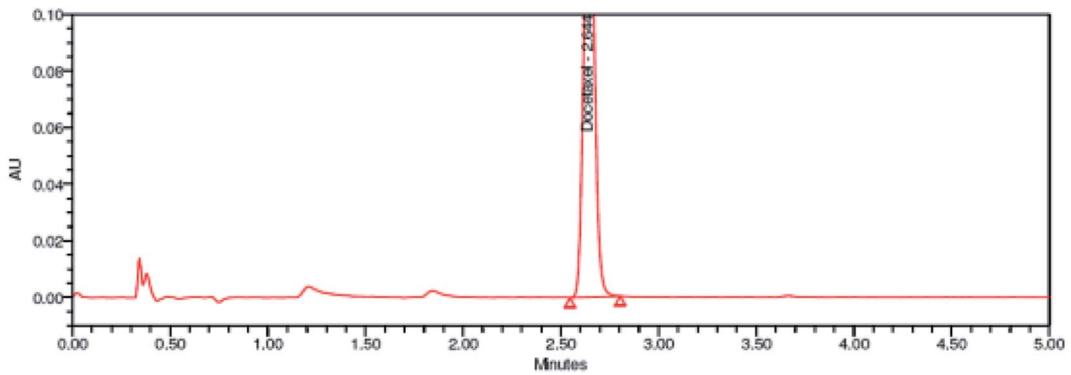

Fig. 20 Sample chromatogram light degradation.

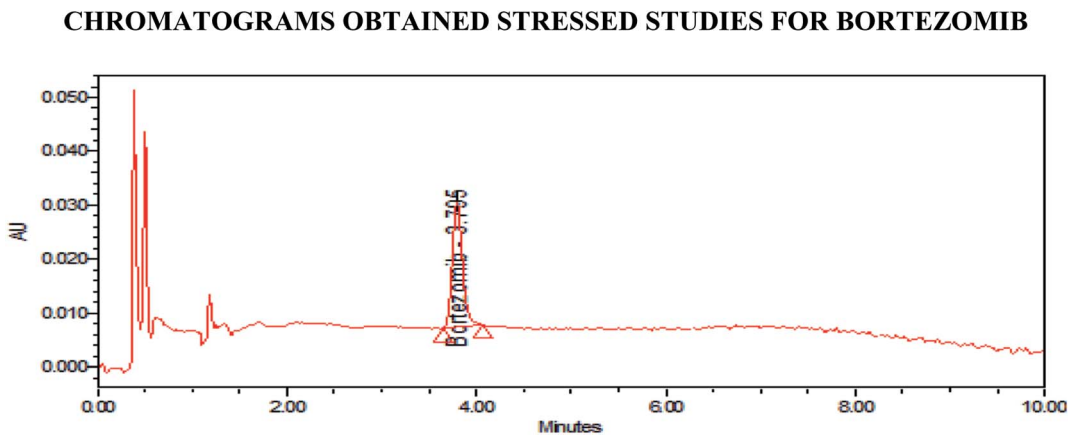

Fig. 21 Acid stressed sample chromatogram.

resolved from the bortezomib peak. The development chromatogram obtained from Trial 4 is shown in Fig. 11.

3.2 Method optimization

Furthermore, the method included optimization studies which were conducted to ensure method repeatability and reproducibility. The optimization studies were conducted by changing the column temperature (40 °C, and 50 °C) and flow rate (1.0, 1.1, 1.2, 1.5 mL min^{-1}),and by adding TFA (0.1%, 0.2%, 0.5%) to organic modifiers for sharp peaks. The optimized chromatogram obtained is shown in Fig. 12.

3.3 Method validation

The optimized methods were fully validated as per the requirements of current ICH guidelines for validation of analytical procedures, *i.e.*, Q2 (R1).[44] The detailed validation experiments and results are discussed below.

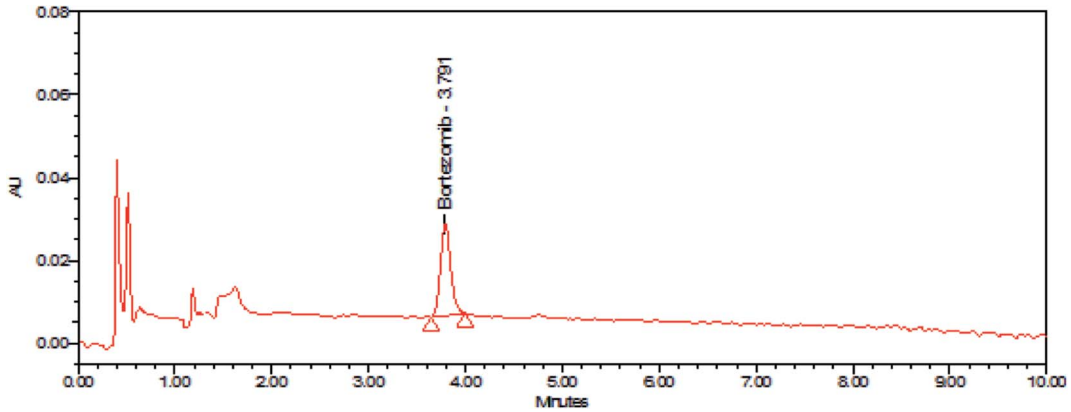

Fig. 22 Base stressed sample chromatogram.

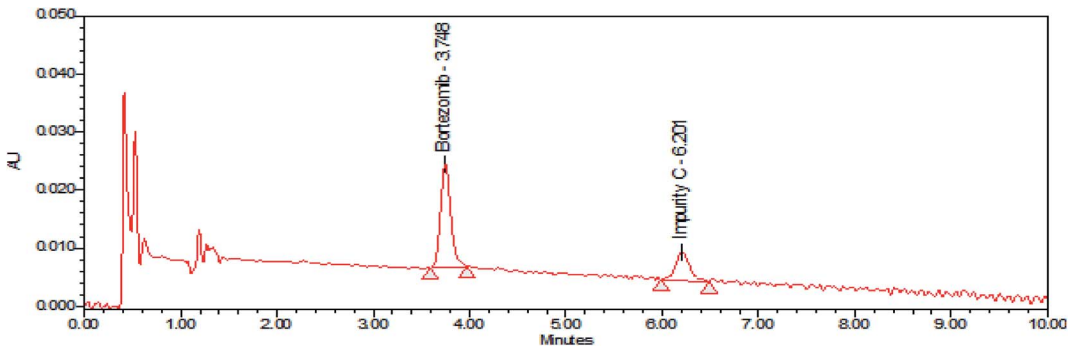

Fig. 23 Peroxide stressed sample chromatogram.

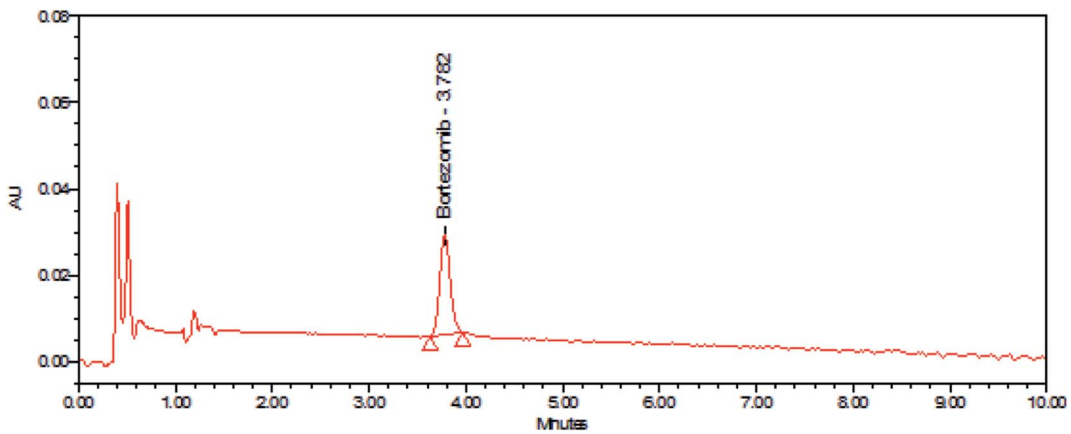

Fig. 24 Thermal stressed sample chromatogram.

3.3.1 System suitability. System suitability parameters were measured to verify the system performance for the intended analysis. For this purpose the USP identification reference standard (CRS) was prepared and injected into the chromatograph (for docetaxel). The USP system suitability parameters *i.e.*, USP plate count, USP tailing, and USP resolution were evaluated. The chromatogram obtained from the system suitability solution, and the peak purity data are shown in Fig. 3. The retention time of docetaxel was 2.679 minutes. Docetaxel was separated from its closest impurity (RT = 2.513) with a resolution of 1.7 (minimum criteria for resolution as per USP is 1.5). The USP plate count was 11 772 and the USP tailing factor was 1.1 for the docetaxel peak.

For bortezomib the system suitability was determined from six replicate injections of bortezomib standard preparation and spiked sample preparation. The resolution between bortezomib

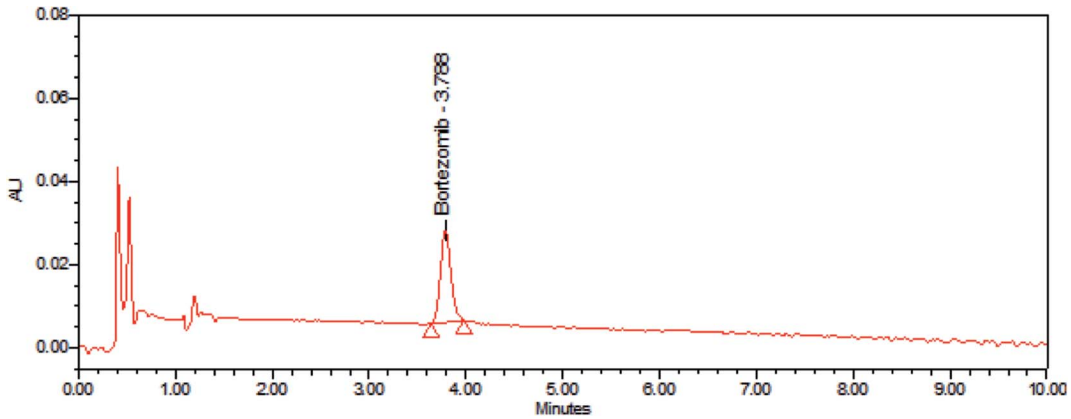

Fig. 25 Sample chromatogram UV degradation.

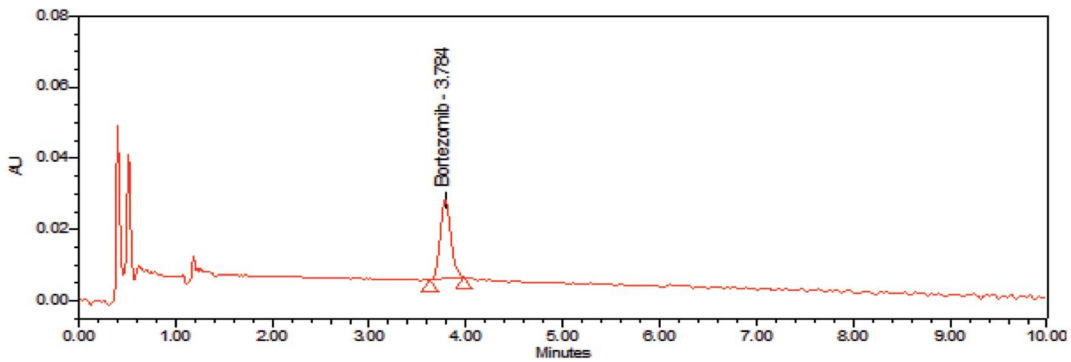

Fig. 26 Sample chromatogram light degradation.

Table 2 Precision data for docetaxel and bortezomib

S. no.	Docetaxel		Bortezomib	
Sample number	Method precision	Intermediate precision	Method precision	Intermediate precision
1	99.1	99.7	99.4	98.7
2	99.7	100.7	98.6	98.9
3	98.9	99.3	99.3	98.9
4	99.0	100.2	99.9	99.3
5	99.5	100.8	99.0	99.0
6	98.5	98.3	99.4	98.4
Mean	99.1	99.8	99.3	98.9
SD	0.43	0.95	0.44	0.30
% RSD	0.4	0.9	0.4	0.3
Overall % RSD (% RSD of 12 measurements)	0.80		0.42	

Table 3 Linearity data for docetaxel and bortezomib

Statistics		Docetaxel	Bortezomib
Linearity	Correlation	0.9999	0.9999
	Slope	666.24	1133.4
	Intercept	−4478.0264	−962.03

and its closely eluting peak was achieved to be 2.4. USP tailing and plate counts for the bortezomib peak were 1.2 and 5662 respectively. The % RSD for six replicate standard injections was 1.3.

Typical system suitability solutions for docetaxel and bortezomib are shown in Fig. 13 and 14 respectively.

3.3.2 Specificity. The specificity of the method was determined to evaluate the interference due to diluent, placebo, and specified impurities, by injecting the diluent (blank), placebo, spiked sample and stress samples. There was no interference found at the retention time of docetaxel and bortezomib due to diluent, placebo, specified impurities and other degradants from the stress samples at the retention time of docetaxel and bortezomib. Furthermore, the homogeneity of the peaks was confirmed from the peak purity data. The content of docetaxel and bortezomib in the presence of their potential impurities was found to be 99.0 and 100.3, respectively. The forced degradation chromatograms are shown in Fig. 15–26.

3.3.3 Precision (repeatability). Precision of a method is a measure of repeatability. It is performed in two ways, method precision and intermediate precision. Method precision was performed by preparing six replicate samples and injecting one after the other, under the conditions as similar as possible, and two injections were taken from each sample. Intermediate precision was performed similar to the method precision on a different day, using different chemicals and columns. The response of docetaxel and bortezomib was measured and the relative standard deviation was calculated. Table 2 shows the precision data for docetaxel and bortezomib. The overall % RSD

Table 4 Accuracy data for docetaxel and bortezomib

Accuracy	Docetaxel			Bortezomib		
Levels	Level-1 (50%)	Level-2 (100%)	Level-3 (150%)	Level-1 (50%)	Level-2 (100%)	Level-3 (150%)
Sample-1	99.1	99.8	99.6	98.3	100.3	99.6
Sample-2	98.7	99.8	99.4	98.9	99.7	99.8
Sample-3	98.7	99.6	98.9	98.3	100.0	99.6
Mean recovery	98.8	99.7	99.3	98.5	100.0	99.7
% RSD	0.2	0.1	0.4	0.4	0.3	0.1

Table 5 Robustness and method sensitivity data for method 1 (docetaxel injection)

S. no.	Condition	RT of docetaxel (minutes)	Assay of docetaxel in spiked sample	USP resolution	USP tailing	% RSD of standard	Method sensitivity yes/no
1	Control (no change)	2.679	99.1	1.7	1.1	0.5	NA[a]
2	Flow (+)1.65 mL min^{-1}	2.415	98.9	1.5	1.1	0.3	Yes
3	Flow (−)1.35 mL min^{-1}	3.250	99.0	1.8	1.1	0.7	Yes
4	Temperature (+)50 °C	2.558	99.0	1.7	1.1	0.4	No
5	Active back pressure regulator (+)2000 psi	2.600	99.0	1.7	1.1	0.4	No
6	Active back pressure regulator (−)1900 psi	2.55	98.9	1.6	1.1	0.4	Yes

[a] Not applicable.

Table 6 Robustness and method sensitivity data for method 2 (bortezomib for injection)

S. no.	Condition	RT of bortezomib (minutes)	Assay of bortezomib in spiked sample	USP resolution	USP tailing	% RSD of standard	Method sensitivity yes/no
1	Control (no change)	3.847	99.3	2.4	1.2	0.8	NA[a]
2	Flow (+)1.43 mL min^{-1}	3.058	99.1	1.8	1.2	1.0	Yes
3	Flow (−)1.17 mL min^{-1}	4.215	99.1	2.5	1.2	1.0	No
4	Column oven temp (+)50 °C	3.812	99.1	2.5	1.2	1.0	Yes
5	Active back pressure regulator (+)2000	3.999	99.0	2.5	1.2	0.8	No
6	Active back pressure regulator (+)1900 psi	3.999	99.0	2.5	1.2	0.8	No

[a] Not applicable.

of 0.80 for docetaxel and 0.42 for bortezomib shows that the methods are precise.

3.3.4 Linearity. The linearity of the method was established in the concentration range of 246.5–1479.18 μg mL^{-1} (*i.e.*, 25% to 150% of the target concentration) for docetaxel and 29.0–290.1 μg mL^{-1} (15% to 160% of the target concentration) for bortezomib. The linear regression data from the calibration plot were indicative of an excellent linear relationship between the peak area and concentration over the range specified above and the data are provided in Table 3. A correlation of 0.9999 was obtained for docetaxel and bortezomib, establishing a linear relationship between the concentration and detector response.

3.3.5 Accuracy. The accuracy of an analytical procedure expresses the degree of the closeness of the obtained results with the true values. The accuracy of the method was tested by the recovery procedure. A known amount of the active drug substance was spiked to the placebo and the recovery was estimated. The accuracy of the method was evaluated from 50% to 150% of target sample concentrations for docetaxel (500 μg mL^{-1}, 1000 μg mL^{-1}, and 1500 μg mL^{-1}), and bortezomib (87 μg mL^{-1}, 175 μg mL^{-1}, and 350 μg mL^{-1}). The average assays were in the range of 98.7 to 99.7 with % RSD 0.1–0.4 for docetaxel and 98.5 to 100.0 with 0.1–0.4% RSD for bortezomib. Table 4 summarizes the accuracy results.

3.3.6 Robustness. The robustness of an analytical method is its capacity to remain unaffected by small but deliberate changes in the method parameters. The robustness of the method is determined by making deliberate variations in the flow rate, column temperature and ABPR (active backpressure regulator). The various altered conditions and the measured system suitability are shown in Tables 5 and 6.

The above robustness data state that an increase in the flow rate resulted in significant variations in the USP resolution and could influence the system suitability results. The column temperature and ABPR have no significant impact on the results.

4 Conclusions

The newly developed CO_2 based assay methods for docetaxel and bortezomib are specific, precise, accurate, linear, and robust. The results obtained from the validation studies were satisfactory and they exhibit excellent performance in terms of sensitivity, speed and cost-effective. This technology being a green technology is environmentally friendly in terms of the waste generated from the system. The method can successfully be employed for high accuracy routine assay testing and release production batches.

Acknowledgements

The authors wish to thank the management of Mylan Laboratories Ltd for supporting this work. The authors also like to thank colleagues in the Analytical development services for their co-operation in carrying out this work.

References

1 N. Budisa and D. Schulze-Makuch, *Life*, 2014, **4**(3), 331–340.
2 M. Koel and M. Kaljurand, *Pure Appl. Chem.*, 2006, **78**(11), 1993–2002; C. A. Eckert, *Nature*, 1996, **383**, 313–318.
3 P. J. Cormier, R. M. Clarke, R. M. McFadden and K. Ghandi, *J. Am. Chem. Soc.*, 2014, **136**, 2200–2203.
4 T. J. Matsuda, *Biosci. Bioeng.*, 2013, **115**, 233–241.
5 L. T. Taylor, *J. Supercrit. Fluids*, **47**, 566.
6 Over View on Acquity UPC^2 System http://www.waters.com/waters/en_US/ACQUITY-UPC2/nav.htm?cid=134658367.
7 V. N. Ganipisetty, D. Jalandhar, R. Bandari, P. Manoj and D. Dhananjay, *Anal. Methods*, 2013, **5**(24), 7008–7013.
8 G. V. Rao, G. Gnanadev, B. Ravi, D. Dhananjaya, P. Manoj, B. Indu and R. V. Nadh, *Anal. Methods*, 2013, **5**(18), 4832–4837.
9 V. N. Ganipisetty, B. Ravi, C. R. Reddy, R. Gurjar, P. Manoj, R. V. Nadh and G. dev Gudipati, *Anal. Methods*, 2015, **7**(3), 1092–1097.
10 A. Polyzos, C. Kosmas, H. Toufexi, N. Malamos, A. Lagadas, C. Kosmidis, P. Ginopoulos, N. Ziras, K. Kandilis and V. Georgoulias, *Anticancer Res.*, 2005, **25**(5), 3559–3564.
11 K. N. Syrigos, I. Dannos, G. Dionellis, I. Bofos, C. Alamara, E. Dimakou, G. Stratakos and S. Papiris, *Anticancer Res.*, 2005, **25**(5), 3489–3493.
12 Y. Kogashiwa, H. Sakurai, T. Kimura and N. Kohno, Docetaxel suppresses invasiveness of head and neck cancer cells *in vitro*, *Cancer Sci.*, 2010, **101**(6), 1382–1386.
13 W. Saif Muhammad, K. Syrinos, R. Penney and K. Kaley, *Anticancer Res.*, 2010, **30**(7), 2905–2909.
14 S. Naito, T. Tsukamoto, H. Koga, T. Harabayashi, Y. Sumiyoshi, S. Hoshi and H. Akaza, *J. Clin. Oncol.*, 2008, **38**(5), 365–372.
15 L. A. Trissel, *Handbook on Injectables*, 14th edn, 2006, pp. 555–562.
16 RxList, The internet drug index, http://www.rxlist.com/taxotere-drug.htm.
17 Velcade, Bortezomib, official website, http://www.velcade.com.
18 Z. Zeng, J. Lin and J. Chen, *Ann. Hematol.*, 2013, **92**(7), 935–943.
19 M. Philippe and T. Cyrille, Initial Treatment of Transplant Candidates with Multiple Myeloma, *Semin. Oncol.*, 2013, **40**(5), 585–591.
20 P. F. Bross, R. Kane, A. T. Farrell, S. Abraham, K. Benson, M. E. Brower, S. Bradley, J. V. Gobburu, A. Goheer, S. L. Lee and J. Leighton, *Clin. Cancer Res.*, 2004, **10**(12), 3954–3964.
21 *USP Monographs: Docetaxel and Docetaxel Injection*, USP39–NF34, 2017, pp. 3564–3567.
22 *Ph. Eur. Monograph: Docetaxel*, 8th edn, 2014, vol. 2, p. 2449.
23 *Indian Pharmacopoeia Monographs: Docetaxel and Docetaxel Injection*, 2014, vol. II, pp. 1608–1610.
24 P. Du, N. Li, H. Wang, S. Yang, Y. Song, X. Han and Y. Shi, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2013, **926**, 101–107.
25 W. Hou, J. W. Watters and H. L. McLeod, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2004, **804**(2), 263–267.
26 H. Yamaguchi, A. Fujikawa, H. Ito, N. Tanaka, A. Furugen, K. Miyamori, N. Takahashi, J. Ogura, M. Kobayashi,

T. Yamada and N. Mano, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2012, **893**, 157–161.
27 I. E. Kuppens, M. J. Van Maanen, H. Rosing, J. H. Schellens and J. H. Beijnen, *Biomed. Chromatogr.*, 2005, **19**(5), 355–361.
28 X. Zhao, Y. Zhao, L. Geng, X. Li, X. Wang, Z. Liu, D. Wang, K. Bi and X. Chen, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2011, **879**(31), 3721–3727.
29 I. E. Kuppens, M. J. van Maanen, H. Rosing, J. H. Schellens and J. H. Beijnen, *Biomed. Chromatogr.*, 2005, **19**, 355–361.
30 J. Guitton, S. Cohen, B. Tranchand, B. Vignal, J. P. Droz, M. Guillaumont, *et al.*, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 2419–2426.
31 K. Chandra Sekhar, P. Sudhakar, G. Ramesh reddy, P. Vijaya Babu and N. Linga swamy, *Int. J. Pharma Bio Sci.*, 2013, **3**, 623–627.
32 K. Arun Kumar and K. Kuna Jagadeesh, *J. Pharm. Res.*, 2012, **5**(6), 3193–3195.
33 S. K. Agrawal, A. Sharma and D. S. Rathore, *World J. Pharm. Pharm. Sci.*, 2014, **3**(4), 1208–1217.
34 M. Utage and B. M. V. Swamy, *Int. J. Pharm. Res. Scholars*, 2013, **2**(2), 27–32.
35 C. Rambabu, S. Venkatrao, G. Ramu and M. Ganesh, *Int. J. Pharm. Pharm. Sci.*, 2011, **3**(3), 303–305.
36 V. V. S. S. Appala Raju, A. Bin Mohamud, P. Janaki Pathi and N. Appala Raju, *Int. J. Pharm.*, 2014, **3**(1), 269–272.
37 C. K. Sekhar, P. Sudhakar, G. R. Reddy, P. V. Babu and N. L. Swamy, *Int. J. Pharma Bio Sci.*, 2013, **3**, 623–627.
38 M. N. Brinda, V. K. Reddy, E. S. Goud and K. V. Kumar, *World J. Pharm. Pharm. Sci.*, 2014, **3**(2), 2521–2529.
39 J. Clemens, M. Longo, A. Seckinger, D. Hose, W. E. Haefeli, J. Weiss and J. A. Burhenne, *J. Chromatogr. A*, 2014, **1345**, 128–138.
40 I. Andriamanana, I. Gana, B. Duretz and A. Hulin, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2013, **926**, 83–91.
41 J. A. Blackwell, R. W. Stringham and J. D. Weckwerth, *Anal. Chem.*, 1997, **69**(3), 409–415.
42 T. A. Berger and J. F. Deye, *ACS Symp. Ser.*, 1992, **488**, 132–142.
43 Selected Papers from the II, Iberoamerican Conference on Supercritical Fluids, *J. Supercrit. Fluids* **56**, 3, 223–330.
44 Validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization of Technical requirements for registration of Pharmaceuticals for human use, Current Step 4 version, November 2005.

