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Original article

An eco-friendly HPLC-UV method for the determination of risedronate in its bulk and tablet dosage form with application to content uniformity, dissolution and stability testing

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ABSTRACT

Risedronate is a nitrogen-containing bisphosphonate for the treatment and prevention of post-menopausal osteoporosis. The current work aims to develop a novel green HPLC-UV method for the rapid analysis of risedronate sodium in bulk and tablet formulation. The analyzed samples were separated on Waters Atlantis dC18 (150 mm × 3.9 mm; 5 µm) column using a green mobile phase consisting of potassium phosphate buffer pH 2.9 and potassium edetate buffer pH 9.5 in a ratio of 1:2, the final pH was adjusted to 6.8 with phosphoric acid, the mobile phase was pumped at a rate of 1.0 mL/min, with column temperature set at 30 °C, eluted samples were detected at 263 nm and the chromatographic run time was 3.0 min. The method was found to be linear over the concentration range of 14–140 µg/mL with a correlation coefficient (r^2) of 0.9994. Accuracy and precision were evaluated from three QC samples (LQC, MQC and HQC) together with the five calibrators where the percentage accuracy was found to be 101.84%. Processed quality control samples of risedronate sodium were tested for stability at different conditions, short term, long term and freeze- thaw stability. The current method was further extended to study the content uniformity of Actonel® tablets following United States Pharmacopoeia (USP) guidelines. The proposed method was fully validated as per ICH guidelines.

1. Introduction

The enthusiasm for the field of green science is developing significantly and is transforming into a fabulous test for researchers to develop new items, methodology and services that achieve the key social, efficient and natural targets as a result of an extended consciousness of environmental prosperity, checking natural contamination, economical modern biology and cleaner creation innovations around the world (Haq et al., 2017). Numerous solvents utilized in the scientific strategies are unpredictable natural mixes of volatile organic compounds (VOCs), that are perilous air poisons (HAPs), combustible, poisonous as well as cancer-causing [e.g., most of analytical techniques accredited by the US Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) utilize destructive and lethal synthetic substances, with no different available alternatives right now] (Garrigues et al., 2010). They additionally present genuine ecological, wellbeing, and security (EHS) concerns, including human and eco-poisonous quality issues, process safety dangers, and waste handling issues.

Abbreviations: HPLC-UV, High-pressure liquid chromatography-ultra violet detection; DAD, Diode Array Detector; LQC, Low quality control; MQC, Medium quality control; HQC, High quality control; % CV, The coefficient of variation; SD, Standard deviation; RSD, Relative standard deviation; r^2, Coefficient of correlation; k, Capacity factor; T_f, USP tailing factor; Rt, Retention time; *N*, Number of theoretical plates; SST, System suitability study; USP, United States Pharmacopoeia; USFDA, US Food and Drug Administration; NaOH, Sodium hydroxide; °C, Degree Celsius; µL, Microlitre; AV, Acceptance value; RT, Room temperature.

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Bisphosphonates are a class of medicinally active chemical compounds, which inhibit osteoclast activity and bone resorption. These compounds were initially synthesized in the 19th century, and were utilized as water softeners (Russell and Rogers, 1999). Clinically, bisphosphonates are used to treat osteoporosis, Paget's disease, bone metastasis, and other conditions with bone fragility. Non-N-containing members include clodronate, etidronate, and tiludronate, whereas N-containing bisphosphonates include neridronate, risedronate, pamidronate, olpadronate, ibandronate, alendronate, and zoledronate (Zacharis and Tzanavaras, 2008). Among the bisphosphonates, alendronate and risedronate are the most widely recognized first-line drugs (Fleisch, 2002; Seo et al., 2014).

Risedronate (1-hydroxy-2-(3-pyridinyl) ethylidine bisphosphonic acid monosodium salt) is a third-generation nitrogen-containing bisphosphonate, approved in October 2010 by the US Food and Drug Administration (USFDA) for the treatment and prevention of postmenopausal osteoporosis (Seo et al., 2014). It is one of the most common first-line medications in the treatment of osteoporosis, Paget's disease, and other conditions of bone fragility since it prevents osteoclast-mediated bone resorption and controls bone metabolism (Siris et al., 1998; Mitchell et al., 1999; Geusens and McClung, 2001). It is marketed in tablet form for oral use in the form of the hemi-pentahydrate, as shown in Fig. 1.

A variety of analytical techniques were utilized for determination risedronate either in pharmaceutical formulations or in different and biological fluids, these includes, capillary electrophoresis (Sun et al., 2013), LC-MS (Zhu et al., 2006; Ghassabian et al., 2012; Bertolini et al., 2014), spectrophotometry (Walash et al., 2008; Walash et al., 2009) and HPLC with UV detection (Vallano et al., 2003; Aluoch et al., 2004; Jia et al., 2006; Kyriakides and Panderi, 2007; Walash et al., 2010).

Analytical techniques develop day after day in a trial to build up new methods that are faster, simple, economic, consistent, reproducible, accurate, precise and most of all eco-friendly in comparison to previous ones.

The objective of this study was to develop and validate a precise, accurate and a high-throughput novel green HPLC-UV method for the fast determination of risedronate sodium in bulk with application to its tablet dosage form (Actonel® tablet). Because risedronate sodium has no derivatizable functional groups and is not easily detected utilizing mass spectrometry technique (Seo et al., 2014), an HPLC-UV assay was developed and defined in this study for the determination of risedronate in bulk as well as in dosage form based on the native UV absorbance of the compound. The proposed method utilizes a 100% aqueous mobile phase with no organic solvent, which reflects the greenness of the method and counts for its added value for risedronate analysis.

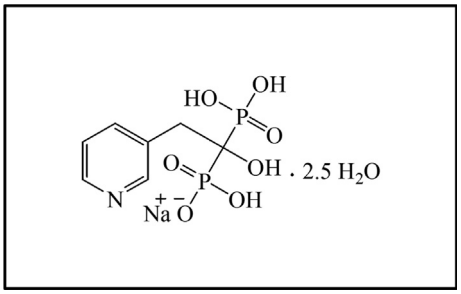

Fig. 1. Chemical structure of risedronate sodium.

2. Materials and methods

2.1. Instrumentation

The chromatographic analysis was performed utilizing an Agilent 1200 series HPLC system (Santa Clara, USA) equipped with a quaternary pump an autosampler (Agilent 1200, USA) (model G1329A), and a Waters Atlantis dC18 (150 cm × 3.9 mm) 5 μm column (Waters, Belgium). The operating temperature of the column was set at 30 °C. The system was equipped with a Diode Array Detector (DAD) set at 263 nm. The liquid chromatography instrument was interfaced with a computer running Agilent B.04.01 ChemStation 32 software under Microsoft Windows XP Professional operating environment.

2.2. Chemicals and reagents

Risedronate sodium hemi-pentahydrate of pharmaceutical purity grade was obtained from Changzhou Huasheng Fine Chemical Co. Ltd. (Changzhou, China), with a certified purity of ≥99.22%. Risedronate sodium tablets (Actonel®), products of Procter & Gamble for Sanofi-Aventis (LLC, USA) each tablet labeled to contain 35.0 mg of risedronate sodium for oral use.

All chemicals and reagents utilized in this research were of analytical grade. Potassium phosphate (monobasic), potassium edetate, phosphoric acid, and sodium hydroxide were purchased from Sigma (St Louis, MO, USA). De-ionized water was used for all preparations. Deionized water was generated in-house using a Millipore Milli-Q Plus system (Billerica, MA, USA).

2.3. Chromatographic conditions

2.3.1. Mobile phase

The green mobile phase buffer mixture consisted of potassium phosphate buffer (pH 2.9) and potassium edetate buffer (pH 9.5) in a ratio of 2:1 adjusted to pH 6.8.

First, potassium phosphate buffer was prepared by dissolving 0.03 g of potassium phosphate monobasic in 250 mL de-ionized water and the pH was adjusted to 2.9 with 150 μL phosphoric acid. Then, potassium edetate buffer was prepared by dissolving 1.59 g of potassium edetate in 1L de-ionized water and adjusted the pH to 9.5 with 5 mL of 1 M NaOH and drops of 5 N NaOH. A volume of 250 mL of potassium phosphate buffer (pH 2.9) and 500 mL of potassium edetate buffer (pH 9.5) were added to get a solution of pH 7.0. Finally, phosphoric acid (20 μL) was added dropwise to reach pH of 6.8. The mobile phase was filtered through 0.45 μm membrane filters and degassed by sonication for 15 min, prior to its use.

2.3.2. HPLC conditions

The analysis was carried out on an Agilent 1200 series HPLC system, using an analytical column Waters Atlantis dC18 (150 mm × 3.9 mm; 5 μm) with a detection wavelength of 263 nm. The operating temperature of the column was set at 30 °C. The injection volume was 20 μL, and the flow rate was maintained at 1.0 mL/min with a total run time of 3 min.

2.4. Method development and validation

2.4.1. Preparation of standard solution and quality control samples

The standard stock solution of risedronate sodium reference standard was prepared by dissolving 14 mg of risedronate sodium in 100 mL of deionized water to get a final concentration of 140 μg/mL. The standard stock solution of risedronate sodium (140 μg/mL)

was then further diluted with deionized water to achieve working solutions of 84, 42, 21, and 14 μg/mL.

Quality control samples were prepared at concentrations of 17.5 μg/mL (low quality control, LQC), 35 μg/mL (medium quality control, MQC) and 70 μg/mL (high quality control, HQC) for risedronate sodium by diluting standard stock solution of risedronate sodium (140 μg/mL) with deionized water.

2.4.2. Linearity

Five calibrators over a concentration range of 14–140 μg/mL (14, 21, 42, 84 and 140 μg/mL) from risedronate were prepared to set the standard calibration curves. The data of peak area versus drug concentration were analyzed by linear least square regression analysis. The correlation coefficient (r^2) was calculated and found to be 0.9993.

2.4.3. Precision and accuracy

Accuracy and precision were assessed from three replicates of QC samples at three different concentrations, 17.5, 35 and 70 μg/mL (low, medium and high) together with the five calibrators.

Percentage accuracy was calculated by the following equation:

$$[\text{Calculated concentration}/\text{Nominal concentration}] \times 100 = \%\ \text{Accuracy}$$

2.4.4. Instrument precision

Instrument (HPLC-UV system) precision was also tested by making multiple injections (n = 10) of MQC (35 μg/mL).

2.4.5. System suitability parameters

Testing for System suitability is considered an integral part of many analytical procedures. The tests reinforce the idea that the equipment, electronics, analytical procedures and analyzed samples constitute an integral system that can be evaluated as a whole.

The system suitability was assessed via using six replicate analyses of risedronate at a concentration of 20 μg/mL. The acceptance criterion is ±2% for the percent coefficient of variation (% CV) for the peak area and retention times for risedronate sodium.

2.4.6. Recovery method for dissolution

Three 1 L beakers (labelled 1, 2 and 3) were taken, 500 mL de-aerated water was added to each beaker, then kept in a water bath heated to 37 °C ± 0.5 °C and allowed to equilibrate. An amount of placebo mixture equivalent to single dose of tablet was added to each beaker.

In a 10 mL volumetric flask, 100 mg of standard risedronate were dissolved in 5 mL de-aerated water, then, made up to volume with de-aerated water to produce a solution of 10.0 mg/mL. To the labelled beakers 1, 2 and 3, an amount of the standard stock solution equivalent to 25%, 50%, and 105% (0.875 mL, 1.75 mL and 3.675 mL), respectively of the label amount was added and mixed well.

Aliquots of 5.0 mL were withdrawn from the dissolution medium from each beaker and filtered through 0.45 μm membrane filter. An appropriate volume of each aliquot was injected into HPLC in triplicate and used to back calculate the injected recovery samples against a calibration curve

2.4.7. Stability studies

The stability of the risedronate in working solution samples were evaluated by analyzing three concentration levels of QC samples (17.5, 35 and 70 μg/ml) under five distinct conditions. The short-term stability was determined by analyzing three QC samples set at room temperature for 6 and 24 h. The long-term stability was evaluated after the three QC samples were stored at −24 °C for 30 days. The freeze-thaw stability of two QC samples (MQC and HQC) were assessed following three freeze–thaw cycles (freezing at −24 °C then thawing at room temperature (RT) for one hour as one cycle). Autosampler stability was determined by analyzing QC samples kept under the autosampler conditions at 4 °C for up to 12 h. Autosampler stability was done with five replicates. All the stability studies were done with three replicates.

2.5. Assay procedure for the determination of risedronate sodium in Actonel® tablets

2.5.1. Preparation of standard solution

Accurately weighed 140 mg of risedronate sodium reference standard were transferred to 100 mL volumetric and diluted to 100 mL with de-ionized water (1.4 mg/mL risedronate sodium).

Working standard solution was prepared by diluting stock solution 10 times, where 5.0 mL of the stock solution were transferred to a 50.0 mL volumetric flask and made up to volume with mobile phase (0.14 mg/mL). An aliquot of 20 μL of the working standard solution was injected five times and peak area was calculated each time.

2.5.2. Preparation of test solution

Ten Actonel® tablets were transferred to a 250 mL volumetric flask, a volume of 150 mL of mobile phase was added, shaken for 10 min and then sonicated for a minimum of 5 min. The solution was then cooled to room temperature and diluted to volume with mobile phase (1.4 mg/mL). Further dilution of this solution with mobile phase was made to obtain a solution of concentration 0.14 mg/mL. The final solution was then filtered through 0.45 μm membrane filter discarding the first few mL of the filtrate. An aliquot of 20 μL of the final test solution was injected three times and peak area was calculated each time. The risedronate content was calculated using the standard formula shown in the results and discussion (USP).

2.6. Content uniformity testing of risedronate sodium in Actonel® tablets by assay

The test for content uniformity depends on the test of the individual content of drug substance in various individual dosage units to decide if the individual content is within the limit set (USP).

The test was applied for estimating the consistency of tablet content of risedronate utilizing 10 tablets as test samples. Every tablet dissolved and diluted with mobile phase as under 'Procedures for tablets'. The content uniformity for each tablet preparation was calculated according to USP guidelines (USP).

2.6.1. Preparation of standard solution

Risedronate sodium working solution (0.14 mg/mL) was diluted by taking 2 mL of this solution in a 10 mL volumetric flask and made up the volume with mobile phase.

2.6.2. Preparation of test solution

One tablet was transferred to each of ten separate 250 mL volumetric flasks marked (T1-T10). 150 mL of mobile phase was added to each flask and shaken by mechanical means until dissolved and diluted with mobile phase up to 250 mL to get a final concentration of 140 μg/mL. It was then filtered through 0.45 μm membrane filter and the first 5 mL of the filtrate was discarded. An aliquot of this mixture was further diluted by taking 2 mL in a 10 mL volumetric flask and made up to volume with mobile phase. An aliquot of 20 μL of standard solution was separately injected five times to calculate the relative standard deviation and tailing factor. Then, 10 μL of each of the test (T1-10) and stan-

dard solutions (S) were injected one time according to the following sequence:

T1; T2; S; T3; T4; S; T5; T6; S; T7; T8; S; T9; T10; S

The chromatograms were recorded and the responses of peak areas obtained, the RSD were calculated for both standard and test solutions. The percentage of risedronate in each of ten tablets was calculated, then mean % content was calculated.

Calculations for tablet analysis were performed using the following formula:

$$Assay = \frac{A_u \times W_{st} \times D \times \frac{A}{100} \times \left(1 - \frac{LOD}{100}\right) \times Av.Wt. \times 100}{A_{st} \times W_t \times L}$$

A_u = Peak area of test solution (average of 3 injections).
A_{st} = Peak area of standard solution (5 injections).
W_{st} = Weight of risedronate working standard in mg.
A% = Assay percent of risedronate working standard.
LOD = Loss on drying of risedronate working standard.
W_t = weight taken of sample powder in mg.
Av. Wt. = Average weight of tablets in mg.
L = label claim of risedronate per tablet.
D = dilution factor

2.7. Dissolution testing for Actonel® tablets

Tablet dissolution test was performed in a multibath (n = 12) dissolution test system DT8 (Pharma Test, Germany), as per the general methods of United States Pharmacopoeia (USP). USP Apparatus II (Paddle) was utilized, at a stirring speed of 50 rpm, in accordance to the commended range of (50–75 rpm) for this type (Food and Drug Administration and Food and Drug Administration, 1997). Twelve tablets were evaluated for risedronate sodium. Each Actonel® tablet was dissolved in 500 mL of de-aerated water as dissolution medium thermostated at 37.0 ± 0.5 °C. Dissolution samples were collected at 5, 10, 15, 20, 30 and 45 min. At each time point, a 2 mL sample was withdrawn from each vessel using an auto-sampler and replaced by fresh medium. The solutions were filtered through a nylon filter (0.45 μm) into labeled glass tubes and analyzed by HPLC.

The amount of risedronate in the tablets was determined from calibration curve and reported as percentage dissolved.

3. Results and discussion

3.1. Green HPLC method development and optimization

Several physical and chemical properties of risedronate sodium were obtained from the literature. The optimization of chromatographic conditions was performed via studying the effect of changing one parameter while keeping the others fixed. These chromatographic conditions, included detection wavelength, mobile phase, stationary phase, and sample preparation procedure.

For this purpose, a series of trials were performed by varying the ratio of potassium phosphate buffer (pH 2.9) and potassium edetate (pH 9.5) and optimizing the chromatographic conditions on the Waters Atlantis dC18 (150 cm × 3.9 mm; 5 μm) column.

A mobile phase consisting of potassium phosphate buffer (pH 2.9) and potassium edetate (pH 9.5) in the ratio 1: 2 v/v (pH 6.8) with a flow rate of 1 mL/min., injection volume of 20 μL, run time of 3 min. and column temperature of 30 °C detected at wavelength (λ) 263 nm were chosen as the best chromatographic conditions for the entire study. Risedronate was eluted producing a symmetrical peak shape within a minimal analysis time with retention time of about 1.3 min (Fig. 2).

3.2. Method validation

The proposed method was validated as per ICH guidelines.

3.2.1. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are either directly or through mathematical transformation proportional to the concentration of the analyte. This proposed HPLC method was assessed by least-squares linear regression analysis of the calibration curve (Miller, 1991).

Linearity of the method was tested for 5 concentrations of risedronate in a range from 14 –140 μg/mL (Table 1a). Each concentration was injected in triplicate and the mean value of the peak areas was obtained. The regression analyses showed satisfactory correlations (r) of 0.9997 indicating a good linearity of the calibration graph (Table 1b).

The linear regression equation was y = 7.0421x – 4.0178, where y and × represented the relationship between Umax, A and doses.

3.2.2. Precision and Accuracy

Accuracy and precision were evaluated from three replicates of QC samples at three different concentrations, *i.e.*, 17.5, 35 and 70 μg/mL (low, medium and high) together with the five calibrators. The accuracy was found to be 101.84% (Table 1a). Instrument precision was within 1.54% (Table 2).

3.2.3. System suitability test (SST)

Parameters such as USP tailing factor (T_f), retention time (Rt), capacity factor (k), peak asymmetry, peak width, and theoretical plates (N) were calculated along with standard deviation and % CV, and compared against the specifications set for the method (Table 3). These parameters were measured using the reference standards of risedronate sodium. SSTs were determined and compared with the recommended limits in United States Pharmacopeia. The mean Rt, k, peak asymmetry, peak width, N, and T_f, were 1.32, 0.10, 0.71, 0.09, 1163 and 1.47 with CVs of 0.39, 4.41, 1.80, 0.77, 1.29 and 1.49%, respectively. All critical parameters tested met the acceptance criteria on all days.

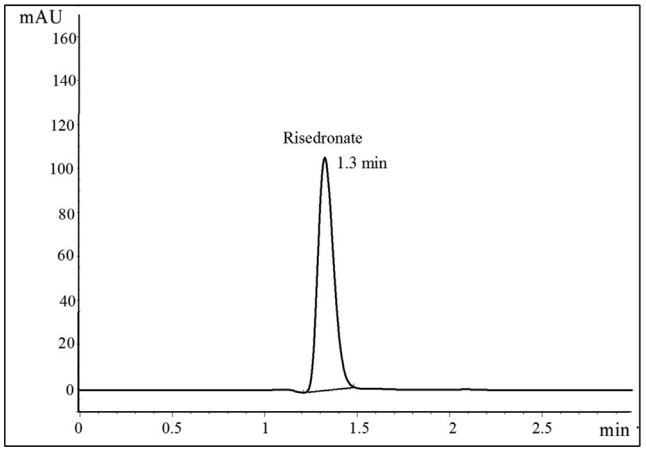

Fig. 2. Chromatogram of risedronate (14 μg/mL) at optimal chromatographic condition.

Table 1a
Risedronate sodium calibrators and QC samples.

	Conc. taken (μg/mL)	Peak area	Conc. Found (μg/mL)	% Recovery
Cal1	14.00	100.20	14.77	105.71
Cal2	21.00	146.85	21.38	102.02
Cal3	42.00	288.65	41.48	98.95
Cal4	84.00	573.93	81.91	97.70
Cal5	140.00	989.94	140.86	100.82
LQC	17.50	127.61	18.65	106.81
MQC	35.00	247.89	35.70	102.21
HQC	70.00	491.53	70.23	100.53
Mean	52.94	370.83	53.12	101.84

n = 3 for each concentration.

Table 1b
Analytical performance data for the determination of risedronate by the proposed chromatographic method.

Parameter	Value
Linearity and range (μg/mL)	14.0–140.0
Correlation coefficient (r)	0.9997
Slope	7.042
Intercept	−4.018
$S_{y/x}$, S.D. of the residuals	9.998
S_a, S.D. of the intercept	7.288
S_b, S.D. of the slope	0.096
S.D.	3.10
% RSD[a]	3.064
% Error[b]	1.385
LOD[c]	3.415
LOQs[d]	10.349

[a] Percentage relative standard deviation.
[b] Percentage relative error.
[c] Limit of detection.
[d] Limit of quantitation.

Table 2
Instrument precision data in dissolution media.

Instrument Precision	
Area of MQC	247.44
	247.35
	247.32
	245.36
	244.70
	245.46
	245.26
	244.50
	244.48
	248.88
Average	246.08
SD	1.54
% CV	0.63

3.2.4. Recovery method for dissolution

The percent recovery of 25%, 50%, and 105% risedronate sodium solutions were found to be 91.6, 94.94 and 97.53%, respectively (Figs. 3A and 3B and Table 4).

3.2.5 . Stability studies

Autosampler stability was assessed by using the processed quality control samples at 4 °C and anticipated batch run time up to 12 h (Table 5). This indicated that the samples were stable for up to 12 h in autosampler at 4 °C without any significant loss. Average stability across concentrations tested was 100.14%. Short term stability indicated that the risedronate in QC samples was stable at room temperature for up to 24 h (Table 6). Long term stability indicated that the drug when kept at −24 °C for a relatively long time and then thawed to room temperature was sufficiently stable at all three concentrations levels tested (Table 7). The average long-term stability at three concentrations was 98.62% with % CV of 8.8%. Table 8 showed the freeze and thaw stability results, which defined the freeze and thaw stability limitations. The practice of 3 freeze-thaw cycles should result in mean difference by <15%. Freeze and thaw stability results indicated that the risedronate working solutions are stable for at least three cycles of freeze and thaw, when stored at −24 °C and thawed to RT. The average freeze-thaw stability at two concentrations was 100.60%.

Table 4
Recovery of risedronate.

Risedronate[a]	Calculated concentration (in μg/ml)	% Recovery	% CV
25%	16.03	91.6	0.67
50%	33.32	94.94	0.43
105%	97.53	97.53	0.11

[a] n = 3 for each concentration.

Table 3
System suitability parameters for risedronate.

Sample	Rt	k factor	Peak asymmetry	Peak width	Plates (N)	T_f
1	1.33	0.11	0.74	0.09	1152	1.41
2	1.32	0.10	0.71	0.09	1182	1.47
3	1.31	0.09	0.70	0.09	1146	1.48
4	1.32	0.10	0.71	0.09	1177	1.48
5	1.31	0.10	0.70	0.09	1174	1.48
6	1.31	0.09	0.71	0.09	1171	1.48
7	1.31	0.10	0.70	0.09	1150	1.48
8	1.32	0.10	0.71	0.09	1150	1.48
9	1.31	0.09	0.70	0.09	1146	1.48
10	1.32	0.10	0.70	0.09	1179	1.49
Average	1.32	0.10	0.71	0.09	1163	1.47
SD	0.005	0.004	0.013	0.001	15.034	0.022
% CV	0.39	4.41	1.80	0.77	1.29	1.49

n = 10 chromatograms for risedronate.

Table 5
Autosampler stability results for risedronate.

Risedronate (μg/mL)	Calculated concentration (μg/ml)[a]		
	Fresh	12 h	% Stability
LQC (17.5)	17.10	17.51	102.40
MQC (35)	35.72	35.4	99.11
HQC (70)	71.00	70.23	98.92

[a] n = 5 at each concentration for each experiment.

Table 7
Long term stability results for risedronate.

Risedronate (μg/mL)	Calculated concentration (μg/mL)[a]		
	Fresh	30 days (−24 °C)	% Stability
LQC (17.5)	17.81	17.07	95.84
MQC (35)	35.20	35.53	100.94
HQC (70)	69.33	68.68	99.10

[a] n = 3 at each concentration and for each experiment.

3.3. Determination of risedronate in the Actonel® tablets

The assay results showed the average percentage risedronate content of 99.80% in Actonel® tablets as shown in Table 9, this counts for the selectivity of the method for determination of risedronate in tablets, where, satisfactory results were obtained with no observed interference. The product Actonel® tablets passed the USP test for assay of dosage units, since the percentage content was between 90 and 110%. The proposed method can be effectively employed for assay of risedronate in Actonel® tablets.

3.4. Content uniformity testing

The simplicity and sensitivity of the proposed methods allowed the uniformity of tablet content for risedronate to be checked according to the USP procedure (USP). The results showed good content uniformity for risedronate in their tablets as shown in Table 10. The product Actonel® tablets passed the USP test for content uniformity of dosage units, since the acceptance value was less than 15. The proposed method can be effectively employed for the content uniformity testing of risedronate in Actonel® tablets.

3.5. Dissolution testing for Actonel® tablets

All the Actonel® tablets showed more than 85% dissolution in 15 min (Table 11, Fig. 4). Therefore, it is classified as "very fast dissolving" tablet. The proposed method can be effectively employed for the dissolution testing of Actonel® tablets.

4. Conclusions

In the present investigation, a new green HPLC-UV method has been developed and validated for the rapid determination of risedronate in Actonel® tablets. The utility of the proposed method was verified by an assay, content uniformity and dissolution testing in commercial Actonel® tablets. The proposed method was found to be accurate, precise and fast for the quantification of risedronate in Actonel® tablets. On comparison to previously reported methods for analysis of risedronate the current method has the advantage of being ecofriendly utilizing a green analysis approach, where the whole analysis procedures are run in aqueous medium, hence, could be applied in a safe manner with no environmental hazard. These results indicated that the proposed method can be successfully employed for a routine analysis of risedronate in bulk and commercial formulations.

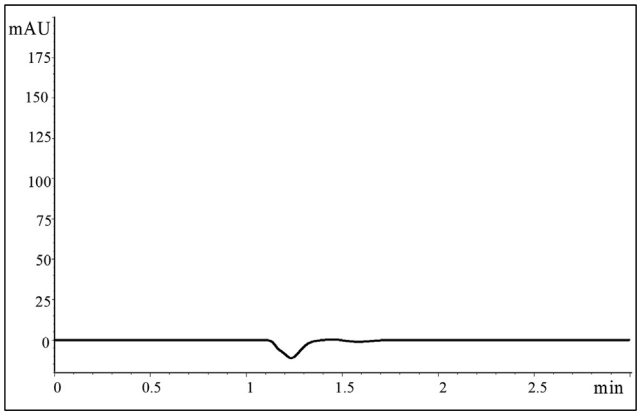

Fig. 3A. Chromatogram of blank dissolution media.

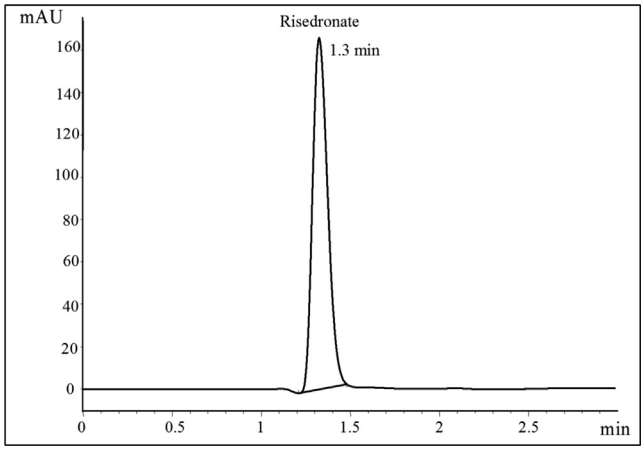

Fig. 3B. Chromatogram of risedronate in dissolution media.

Table 6
Short term stability results for risedronate.

Risedronate (μg/mL)	Calculated concentration (μg/mL)[a]						
	Fresh	6 h (RT)	% Stability	24 h (4 °C)	% Stability	24 h (RT)	% Stability
LQC (17.5)	17.81	17.70	99.40	17.18	96.46	18.00	101.06
MQC (35)	35.20	34.92	99.20	34.45	97.89	34.62	98.35
HQC (70)	69.33	68.82	99.26	70.18	101.22	70.03	101.01

[a] n = 3 at each concentration and for each experiment; RT = room temperature.

Table 8
Freeze and thaw stability results for risedronate.

Risedronate (μg/mL)	Calculated concentration (μg/mL)[a]			
	Fresh	After 3 cycles[a]	% Stability	% CV
MQC (35)	35.21	35.71	101.42	6.0
HQC (70)	69.5	69.35	99.78	8.0

[a] n = 3 at each concentration and for each experiment.

Table 9
Assay determination of risedronate in Actonel® tablets.

Peak area	Standard	Reference
	A_{st}	A_u
1	1706.50	1875.04
2	1711.50	1880.68
3	1712.00	1880.93
4	1712.30	
5	1713.70	
Average area	1711.20	1878.88
SD	2.75	3.33
CV%	0.16	0.18
W_{St} (Weight of risedronate sodium working Standard)	140.00 mg	
A% (assay percent)	99.80	
LOD	13.30	
A_{St} (Standard peak area)	1711.20	
Av. Wt. (Average weight of tablets)		250.61
W_t (wt. of test sample powder)		2506.12
L (label claim)		35.00
A_u(Test peak area)		1878.88
Dilution factor	2.5	

Table 10
Results of content uniformity testing of risedronate in individual Actonel® tablet.

Tablet	Amount of risedronate (mg)	Percentage of the label claim of Actonel® tablets
1	33.76	96.47
2	33.34	95.26
3	32.28	92.23
4	33.36	95.32
5	31.38	89.65
6	33.33	95.24
7	32.53	92.95
8	31.93	91.22
9	34.80	99.43
10	32.40	92.57
Average	32.91	94.03
± SD	1.04	2.98
CV	3.17	3.17
Acceptance value (AV)*		11.62
Maximum allowed value*		15

* (The United States Pharmacopoeia 30, the National Formulary 25, 2007).

Table 11
Mean percent dissolved (±SD) from Actonel® tablets.

Time (in minutes)	Mean percent dissolved[a] (%)	± SD	% CV
0.00	0.00	0.00	0.00
5.00	87.98	5.23	5.95
10.00	93.59	3.25	3.47
15.00	95.05	2.91	3.07
20.00	97.41	1.91	1.97
30.00	99.18	2.48	2.50
45.00	99.84	2.27	2.28

[a] n = 12 Actonel® tablets.

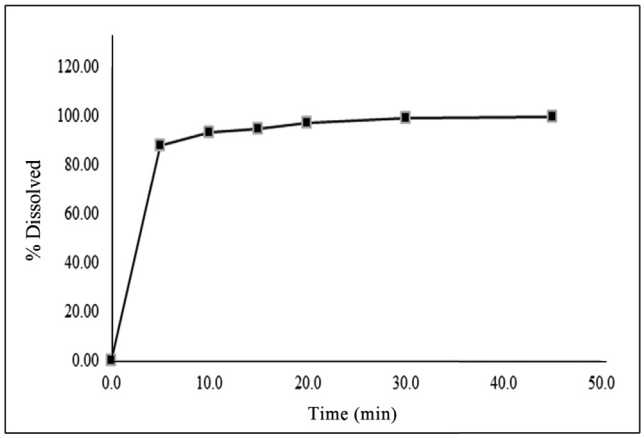

Fig. 4. Dissolution profile of risedronate in Actonel® tablets.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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