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# Development and validation of a reversed-phase ion-pair high-performance liquid chromatographic method for the determination of risedronate in pharmaceutical preparations

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**Abstract**

A stability indicating, reversed-phase ion-pair high-performance liquid chromatographic method was developed and validated for the determination of risedronate in pharmaceutical dosage forms. The determination was performed on a BDS $C_{18}$ analytical column (250 mm × 4.6 mm i.d., 5 μm particle size); the mobile phase consisted of 0.005 M tetrabutylammonium hydroxide and 0.005 M pyrophosphate sodium (pH 7.0) mixed with acetonitrile in a ratio (78:22, v/v) and pumped at a flow rate 1.00 mL $min^{-1}$. The ultraviolet (UV) detector was operated at 262 nm. The retention times of magnesium ascorbyl phosphate, which was used as internal standard and risedronate were 4.94 and 5.95 min, respectively. The calibration graph was ranged from 2.50 to 20.00 μg $mL^{-1}$, while detection and quantitation limits were found to be 0.48 and 1.61 μg $mL^{-1}$, respectively. The intra- and inter-day percentage relative standard deviations, %R.S.D., were less than 5.9%, while the relative percentage error, $\%E_r$, was less than 0.4%. The method was applied to the quality control of commercial tablets and content uniformity test and proved to be suitable for rapid and reliable quality control.
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*Keywords:* Risedronate; Magnesium ascorbyl phosphate; Ion-pair liquid chromatography; Pharmaceutical dosage forms; Stability indicating

## 1. Introduction

Risedronate sodium (1-hydroxy-2-(3-pyridinyl) ethylidene bisphosphonic acid monosodium salt) (Fig. 1), is a new generation pyridinyl bisphosphonate that is used in the treatment of skeletal disorders as it inhibits osteoclast-mediated bone resorption and modulates bone metabolism [1–3]. The enzyme farnesyl pyrophosphate synthase (FPPS), involved in the mevalonate pathway, has been identified as the target of risedronate [4]. In particular, risedronate along with other aromatic nitrogen-containing biphosphonates inhibits the FPPS activity [5]. It is the only osteoporosis medication currently proven to reduce the risk of vertebral fracture and to prevent nonvertebral osteoporosis-related fractures in postmenopausal osteoporotic women [6,7]. Risedronate is also effective in the treatment of Paget's disease of bone [8,9].

Up to now, only a few methods have been developed that focus on the determination of risedronate in biological matrices including gas chromatography–mass spectrometry following acylation and silylation to form a volatile derivative[10] and a very sensitive, yet highly complicated, enzyme linked immunosorbent assay [11]. Literature survey revealed that ion-pair high-performance liquid chromatography (HPLC) has been considered as the technique of choice for the determination of risedronate. In particular, a column-switching ion-pair HPLC method using 1-octyltriethylammonium phosphate as ion-pair agent [12] and an ion-pair HPLC method with tetrabutylammonium bromide as ion-pair agent [13] have been proposed for the determination of risedronate in biological matrices, while an ion-pair HPLC method using tetrabutylammonium phosphate as ion-pair agent has been applied to the determination of risedronate in pharmaceutical preparations [14]. Though the above described ion-pair HPLC methods are successful approaches to the determination of risedronate, the run time of analysis remains extremely long (greater than 12 min) for quality control purposes.

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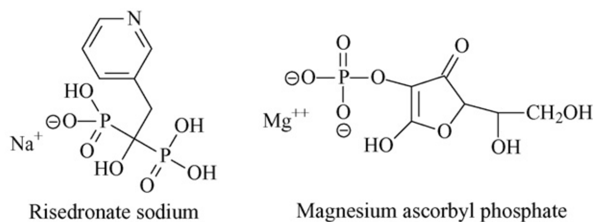


Fig. 1. Chemical structures of risedronate sodium and magnesium ascorbyl phosphate (IS).

To the best of our knowledge no official report for the determination of risedronate sodium in bulk material and tablets has ever been published in any pharmacopoeia. Thus, we thought that it would be of particular interest to develop and validate an improved stability indicating HPLC method for the quality control of risedronate in pharmaceutical preparations that would also reduce the sample throughput. Reversed-phase ion-pair liquid chromatography on a BDS $C_{18}$ analytical column was used to perform the experiments. The chromatographic behaviour of both risedronate and magnesium ascorbyl phosphate [15] (Fig. 1) that was used as the internal standard was thoroughly investigated using tetrabutylammonium hydroxide as ion-pair agent. The proposed method is applicable as well for routine analysis and content uniformity test of risedronate in tablets and complies well with the validation requirements in the pharmaceutical industry.

## 2. Experimental

### 2.1. Equipment

The chromatographic equipment used consisted of a Spectra-Physics SP8810 precision isocratic pump (Darmstadt, Germany) and a Rheodyne 7725i injector (California, CA, USA) with a 10 μL sample loop. Detection was performed by a Waters 486 ultraviolet detector (Milford, MA, USA), using a detection wavelength of 262 nm. Data acquisition was performed using a HP 3394A integrator (Avondale, PA, USA). All pH measurements were performed on a Metrohm 654 pH meter (Herisau, Switzerland). Dilutions were accomplished using Hamilton precision pipettes (Bonaduz, Switzerland).

### 2.2. Materials and reagents

Solvents were of HPLC grade and were purchased from Merck (Darmstadt). Tetrabutylammonium hydroxide solution 40.0% (v/v) in water and glacial acetic acid all of analytical-reagent grade were also purchased from Merck (Darmstadt). Sodium pyrophosphate was obtained from Sigma–Aldrich Inc. (St. Louis, MO, USA). Water was deionised and further purified by means of a Milli-Q Plus Water Purification System, Millipore (Molsheim, France). Risedronate sodium of pharmaceutical purity grade was obtained from Glenmark Pharmaceuticals (Berkshire, UK), while magnesium ascorbyl phosphate was purchased from Sigma–Aldrich.

All substances were used without any further purification. Risedronate tablets are products of Procter & Gamble Pharmaceuticals (NY, USA) and each tablet is labelled to contain 5.0 mg of risedronate sodium. The excipients present in tablets are: lactose monohydrate, microcrystalline cellulose, crospovidone, magnesium stearate, ferric oxide yellow (E172), hypromellose, macrogol 400, hydroxyl-propyl cellulose, macrogol 8000, silicon dioxide and titanium dioxide (E171).

### 2.3. Chromatographic conditions and measurement procedure

The LC column used was a 4.6 mm i.d. × 250 mm length Hypersil BDS $C_{18}$ analytical column that was purchased from Thermo-Hypersil-Keystone (Bellefonte, PA, USA). The mobile phase was consisted of 0.005 M tetrabutylammonium hydroxide and 0.005 M sodium pyrophosphate (pH 7.0) mixed with acetonitrile in a ratio (78:22, v/v). It was filtered through a 0.45 μm nylon-membrane filter obtained from GelmanSciences (Ann Arbor, MI, USA) and degassed under vacuum prior to use. The flow-rate of the mobile phase was maintained at 1 mL $min^{-1}$ with a column inlet pressure of 1350 psi in order to separate risedronate and the internal standard magnesium ascorbyl phosphate. HPLC analysis was conducted at ambient temperature. Peak areas were measured for the quantitation of the analyte.

### 2.4. Stock and working standard solutions

Stock standard solutions of risedronate (Rsd) 500.0 μg $mL^{-1}$ and magnesium ascorbyl phosphate (Map) 250.0 μg $mL^{-1}$ were prepared by dissolving appropriate amounts of the compounds in water. These solutions were stored in the dark under refrigeration at 4 °C and were found to be stable for several weeks.

Calibration standards were prepared over the concentration range of 2.50, 5.00, 10.00, 15.00 and 20.00 μg $mL^{-1}$ for risedronate by appropriate dilutions of the above mentioned stock standard solution in 10.0 mL of water. In each sample 2.50 μg $mL^{-1}$ of the internal standard, Map, was added.

Quality control (QC) samples were also prepared in water at three concentration levels (2.5, 10.0 and 20.0 μg $mL^{-1}$). Separate stock standard solutions of Rsd were used for the preparation of calibration standard solutions and quality control samples. All calibration standards and QC samples were prepared freshly every day and found to be stable during the analysis time.

### 2.5. Assay of pharmaceutical preparations

Twenty tablets were weighed and finely pulverised. An appropriate portion of this powder, equivalent to 5.0 mg of Rsd was placed in a 50 mL volumetric flask with 40 mL of water. The solution was sonicated for 10 min and diluted to volume with water. A portion of this solution was centrifuged at 4000 rev $min^{-1}$ (2890 g) for 10 min. A 1.0 mL aliquot was transferred to a 10 mL volumetric flask and diluted to volume with water. In each sample 2.50 μg $mL^{-1}$ of the internal standard Map was added; 10 μL samples were injected into the HPLC system. Peak area ratios of risedronate to that of the internal

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