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# A stability indicating LC method for Rivastigmine hydrogen tartrate

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**Abstract**

An isocratic, reversed-phase liquid chromatographic (RPLC) method was developed for the quantitative determination of Rivastigmine hydrogen tartrate, a cholinesterase inhibitor in bulk drugs and in pharmaceutical dosage forms. The developed method is also applicable for the related substance determination of Rivastigmine hydrogen tartrate in bulk drugs. The chromatographic separation was achieved on a Waters X Terra RP18 (250 mm × 4.6 mm, 5 μm) column using aqueous 0.01 M sodium-1-heptane sulphonate (pH: 3.0 with dilute phosphoric acid)–acetonitrile (72:28, v/v) as a mobile phase. The chromatographic resolution between Rivastigmine and its potential impurity, namely (*S*)-3-(1-dimethylaminoethyl) phenol (Imp 1) was found to be greater than four. Forced degradation studies were performed for Rivastigmine hydrogen tartrate bulk drug using acid (0.5N hydrochloric acid), base (0.5N sodium hydroxide), oxidation (3% hydrogen peroxide), heat (60 °C) and UV light (254 nm). No degradation was observed for Rivastigmine hydrogen tartrate except in base hydrolysis and the formed degradation product was found to be Imp 1. The mass balance of Rivastigmine hydrogen tartrate was close to 100 in all the stress conditions. The limit of detection (LOD) and limit of quantification (LOQ) of Imp 1 were found to be 100 and 300 ng/ml, respectively, for 10 μl injection volume. The percentage recovery of Imp 1 in bulk drug sample was ranged from 95.2 to 104.3. The active pharmaceutical ingredient was extracted from its finished dosage form (capsule) using water. The percentage recovery of Rivastigmine hydrogen tartrate was ranged from 99.2 to 101.3 and 98.6 to 101.5 in bulk and pharmaceutical formulation samples, respectively. Rivastigmine hydrogen tartrate sample solution and mobile phase were found to be stable for at least 48 h. The developed method was validated with respect to linearity, accuracy, precision, robustness and forced degradation studies prove the stability indicating power of the method.


*Keywords:* Rivastigmine hydrogen tartrate; Alzheimer's disease; RPLC; Forced degradation; Validation; Pharmaceutical dosage form; Solution and mobile phase stability

## 1. Introduction

Alzheimer's disease is a progressive neuro-dogenerative disorder characterized by loss of short-term memory and immediate recall and decline in other cognitive functions such as attention [1,2]. Memory loss eventually becomes so severe that patients with Alzheimer's disease lose the ability to care for themselves. Alzheimer's disease is recognized as being one of the most important challenges facing medicine in the 21st century due to aging population and high cost of managing the disease.

Rivastigmine hydrogen tartrate (Exelon), (−)*S*-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methylphenyl-carbamate hydrogen tartrate, a carbamate inhibitor of acetylcholinesterase is used for the treatment of mild to moderate Alzheimer's disease in adults [3]. Exelon helps to slow down the mental decline that happens in people with Alzheimer's disease and it helps to improve the ability to cope with everyday activities.

Few HPLC methods were reported in the literature for the quantitative determination of Rivastigmine and its major metabolite with atmospheric pressure chemical ionization tandem mass spectroscopy and for the determination of the dissociation constants of basic acetyl cholinesterase inhibitors [4,5].

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So far to our present knowledge, no LC methods were reported for the analysis of Rivastigmine hydrogen tartrate in bulk drugs and in pharmaceutical formulations. It is felt necessary to develop a stability indicating LC method for the related substance determination and quantitative determination of Rivastigmine hydrogen tartrate. This paper deals with the forced degradation of the developed method under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat and UV. This paper also deals with the assay method validation of Rivastigmine and related substances validation for accurate quantification of Imp 1 in Rivastigmine bulk samples.

## 2. Experimental

### 2.1. Chemicals

Samples of Rivastigmine hydrogen tartrate and Imp 1 were received from Process Research Department of Custom Chemical Services of Dr. Reddy's Laboratories Limited, Hyderabad, India. The chemical structures of Rivastigmine hydrogen tartrate and Imp 1 are shown in Fig. 1. Capsules of Exelon (6 mg) were purchased from Novartis Pharma AG, Basel, Switzerland. The excipients present in Exelon capsules are gelatin, iron oxide, magnesium state, methylhydroxypropylcellulose, microcrystalline cellulose and titanium dioxide. HPLC grade acetonitrile was purchased from Merck, Germany. Analytical reagent grade sodium-1-heptane sulphonate was purchased from Lancaster, USA. High pure water was prepared by using Millipore Milli Q plus purification system.

### 2.2. Equipment

The LC system used for method development and forced degradation studies was Agilent 1100 series LC system with a diode array detector. The output signal was monitored and processed using a Chemstation software (Agilent) on Pentium computer (Digital Equipment Co.).

The LC system used for method validation was Waters Alliance consisting of 2695 separation module, 270852 thermostatic compartment and a 2487 dual wavelength detector. The LC system used for intermediate precision study was Waters LCM1 plus consisted of 600 pump, 715 auto injector, 486 tunable absorbance detectors, 270852 thermostatic compartments and an auto sampler. The output signal was monitored and processed using a millennium 32-chromatography manager software (Waters) on Pentium computer (Digital Equipment Co.).

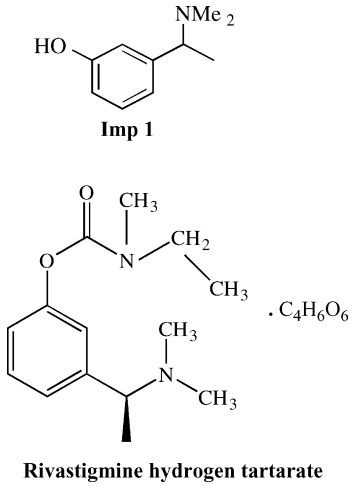


Fig. 1. Chemical structures of Imp 1 and Rivastigmine hydrogen tartrate.

### 2.3. Chromatographic conditions

The chromatographic column used was a Waters 250 mm × 4.6 mm X Terra RP-18 column with 5 μm particles. The mobile phase was aqueous sodium-1-heptane sulphonate (pH: 3.0 adjusted with dilute phosphoric acid: 0.01 M)–acetonitrile (72:28, v/v). The flow rate of the mobile phase was 1.0 ml/min. The column was maintained at 30 °C and the wavelength was monitored at a wavelength of 217 nm. The injection volume was 10 μl. Water was used as a diluent.

### 2.4. Preparation of standard solutions

A stock solution of Rivastigmine hydrogen tartrate (5.0 mg/ml) was prepared by dissolving appropriate amount of substance in water. Working solutions of Rivastigmine hydrogen tartrate 500 and 100 μg/ml were prepared from stock solution for related substances determination and assay determination respectively. A stock solution of Imp 1 (0.5 mg/ml) was prepared in acetonitrile.

### 2.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [6]. The specificity of the developed LC method for Rivastigmine hydrogen tartrate was carried out in the presence of its related potential impurity namely Imp 1.

Forced degradation studies were also performed for Rivastigmine hydrogen tartrate bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254 nm), heat (60 °C), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3% $H_2O_2$) to evaluate the ability of the proposed method to separate Rivastigmine from its degradation products [7]. For heat and light studies, study period was 10 days where as for acid, base and oxidation, it was 48 h. Peak purity test was carried out for Rivastigmine peak by using PDA detector in stress samples. Assay studies were carried out for stress samples against qualified Rivastigmine hydrogen tartrate reference standard and the mass balance (% assay + % degradation) was calcu-

lated. Assay was also calculated for Rivastigmine hydrogen tartrate bulk sample by spiking Imp 1 at the specification level (0.15%). The excipient mixture present in Exelon capsules was injected in the optimized conditions to show the specificity of the method in formulation samples of Rivastigmine.

### 2.6. Method validation

#### 2.6.1. Precision

Assay method precision was evaluated by carrying out six independent assays of Rivastigmine hydrogen tartrate test sample against qualified reference standard and calculating the % R.S.D. of assay.

The precision of the related substance method was checked by injecting six individual preparations of Rivastigmine hydrogen tartrate (0.5 mg/ml) spiked with 0.15% of Imp 1 with respect to Rivastigmine hydrogen tartrate analyte concentration and calculating the % R.S.D. of area for Imp 1.

The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory.

#### 2.6.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for Imp 1 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration [8]. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp 1 and calculating the % R.S.D. of the area.

#### 2.6.3. Linearity

Linearity test solutions for assay method were prepared from Rivastigmine hydrogen tartrate stock solution at six concentration levels from 25 to 150% of assay analyte concentration (25, 50, 75, 100, 125 and 150 μg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis.

Linearity test solutions for related substance method were prepared from the Imp 1 stock solution at six concentration levels from LOQ to 200% of the specification (0.06, 0.075, 0.15, 0.1875, 0.225 and 0.3%). The calibration curve was drawn by plotting the peak area of Imp 1 versus its corresponding concentration.

Linearity was checked for three consecutive days in the same concentration range for both assay and related substance method and calculated the % R.S.D. value of the slope and *Y*-intercept of the calibration curve.

#### 2.6.4. Accuracy

The accuracy of the assay method was evaluated in triplicate at three-concentration levels i.e. 50, 100 and 150 μg/ml in bulk drug sample. The % recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained in Section 2.6.3.

The accuracy of the assay method was also evaluated using Rivastigmine hydrogen tartrate formulation (Exelon 6 mg). Ten capsules of Exelon (6 mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 50 mg of the active pharmaceutical ingredient, was extracted into water in a 100 ml volumetric flask by vortex mixing followed by ultra sonication. The resultant mixture was filtered through 0.45 μm membrane filter. The filtrate was used as stock solution for the preparing accuracy test solutions. Test solutions in triplicate were prepared at the same concentration levels as prepared in bulk drug samples and % recoveries were calculated in the similar manner.

The Rivastigmine hydrogen tartrate bulk sample, provided by Process Research Department of Custom Chemical Services, showed the presence of 0.02% of Imp 1. Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of Imp 1 in bulk drug samples.

The study was carried out in triplicate at 0.075, 0.15 and 0.225% of the Rivastigmine hydrogen tartrate analyte concentration (500 μg/ml). The % recoveries for Imp 1 were calculated from the slope and *Y*-intercept of the calibration curve obtained in Section 2.6.3.

#### 2.6.5. Robustness

To determine the robustness of the developed method experimental conditions were purposely altered and the resolution between Rivastigmine and Imp 1 was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 ml/min while the other mobile phase components were held constant as stated in Section 2.3. The effect of percent organic strength on resolution was studied by varying acetonitrile from −3 to +3% while the other mobile phase components were held constant as stated in Section 2.3. The effect of column temperature on resolution was studied at 25 and 35 °C instead of 30 °C while the other mobile phase components were held constant as stated in Section 2.3.

#### 2.6.6. Solution stability and mobile phase stability

The solution stability of Rivastigmine hydrogen tartrate in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 2 days. The same sample solutions were assayed for 6 h interval up to the study period. Furthermore, mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for 6 h interval up to 2 days. Mobile phase prepared was kept constant during the study period. The % R.S.D. of assay of Rivastigmine was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of Rivastigmine hydrogen tartrate in the related substance method was carried out by leaving

Table 1
System suitability report

| Compound ($n = 3$) | Capacity factor | USP resolution ($R_S$) | USP tailing factor ($T$) | No. of theoretical plates ($N$) tangent method |
|---|---|---|---|---|
| Imp 1 | 3.0 | – | 1.2 | 5500 |
| Rivastigmine | 7.0 | 5.0 | 1.3 | 4000 |

*n*, number of determinations.

sample solution in tightly capped volumetric flask at room temperature for 2 days. Content of Imp 1 was checked for 6 h interval up to the study period. Mobile phase stability was also carried out for 2 days by injecting the freshly prepared sample solutions for 6 h interval. Content of Imp 1 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

## 3. Results and discussion

### 3.1. Optimization of chromatographic conditions

(*S*)-3-(1-dimethylaminoethyl) phenol (Imp 1) was the potential impurity present above 0.1% in Rivastigmine hydrogen tartrate bulk sample produced by Dr. Reddy's laboratories. The main target of the chromatographic method is to get the separation of Rivastigmine hydrogen tartrate and the Imp 1. Rivastigmine and Imp 1 were coeluted by using different stationary phases like C18, C8, Cyno and phenyl and different mobile phases containing buffers like phosphate, sulphate and citrate with different pH 2–9 and using organic modifiers like acetonitrile, methanol and THF in the mobile phase. Introduction of ion-pairing agent (sodium-1-heptane sulphonate) has played a key role in achieving the separation between Imp 1 and Rivastigmine.

The chromatographic separation was achieved using a mobile phase containing a mixture of aqueous 10 mM sodium-1-heptane sulphonate (pH: 3.0 adjusted with dilute phosphoric acid and acetonitrile in the ratio (72:28, v/v)). The column temperature (30 °C) has improved the peak shape of Rivastigmine. In the optimized conditions, Rivastigmine and Imp 1 were separated with a resolution of greater than four and the typical retention times of tartric acid, Imp1 and Rivastigmine were about 2.0, 4.0 and 8.0 min, respectively. The system suitability results were given in Table 1 and the developed LC method was found to be specific for Rivastigmine and its potential impurity Imp 1.

### 3.2. Results of forced degradation experiments

Degradation was not observed for Rivastigmine hydrogen tartrate sample during stress conditions like UV light, heat, acid and oxidation except in base hydrolysis (Fig. 2). Rivastigmine hydrogen tartrate was degraded into Imp 1 during base hydrolysis and it was confirmed by co-injection with qualified Imp 1 standard. Peak purity test results confirm Rivastigmine peak is homogeneous in all the stress conditions tested. The mass balance of Rivastigmine hydrogen tartrate in stress samples was close to 100% and moreover, the unaffected assay of Rivastigmine hydrogen tartrate in the presence of Imp 1 confirms the stability indicating power of the method. The non-interference of excipient peaks with Rivastigmine confirms the specificity of the developed method in formulation samples (Fig. 2). The summary of forced degradation studies is given in Table 2.

### 3.3. Precision

The % R.S.D. of assay of Rivastigmine during assay method precision study was within 1% and the % R.S.D. of area of Imp1 in related substance method precision study was within 5%. The % R.S.D. of assay results obtained in intermediate precision study was within 1% and the % R.S.D. of area of Imp1 was within 6%, confirming good precision of the method.

### 3.4. Limit of detection and limit of quantification

The limit of detection and limit of quantification of Imp 1 were 100 and 300 ng/ml for 10 μl injection volume. The method precision for Imp 1 at LOQ level was below 10%.

### 3.5. Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 25 to 150 μg/ml for

Table 2
Summary of forced degradation results

| Stress condition | Time | Assay of active substance (%) | Mass balance (% assay + % impurity) | Remarks |
|---|---|---|---|---|
| Acid hydrolysis (0.5N HCl) | 48 h | 99.5 | 99.5 | No degradation products formed |
| Base hydrolysis (0.5N NaOH) | 48 h | 97.5 | 99.6 | Rivastigmine hydrogen tartrate was degraded into Imp 1 |
| Oxidation (3% $H_2O_2$) | 48 h | 99.2 | 99.2 | No degradation products formed |
| Thermal (60 °C) | 10 days | 99.4 | 99.4 | No degradation products formed |
| UV (254 nm) | 10 days | 99.3 | 99.3 | No degradation products formed |

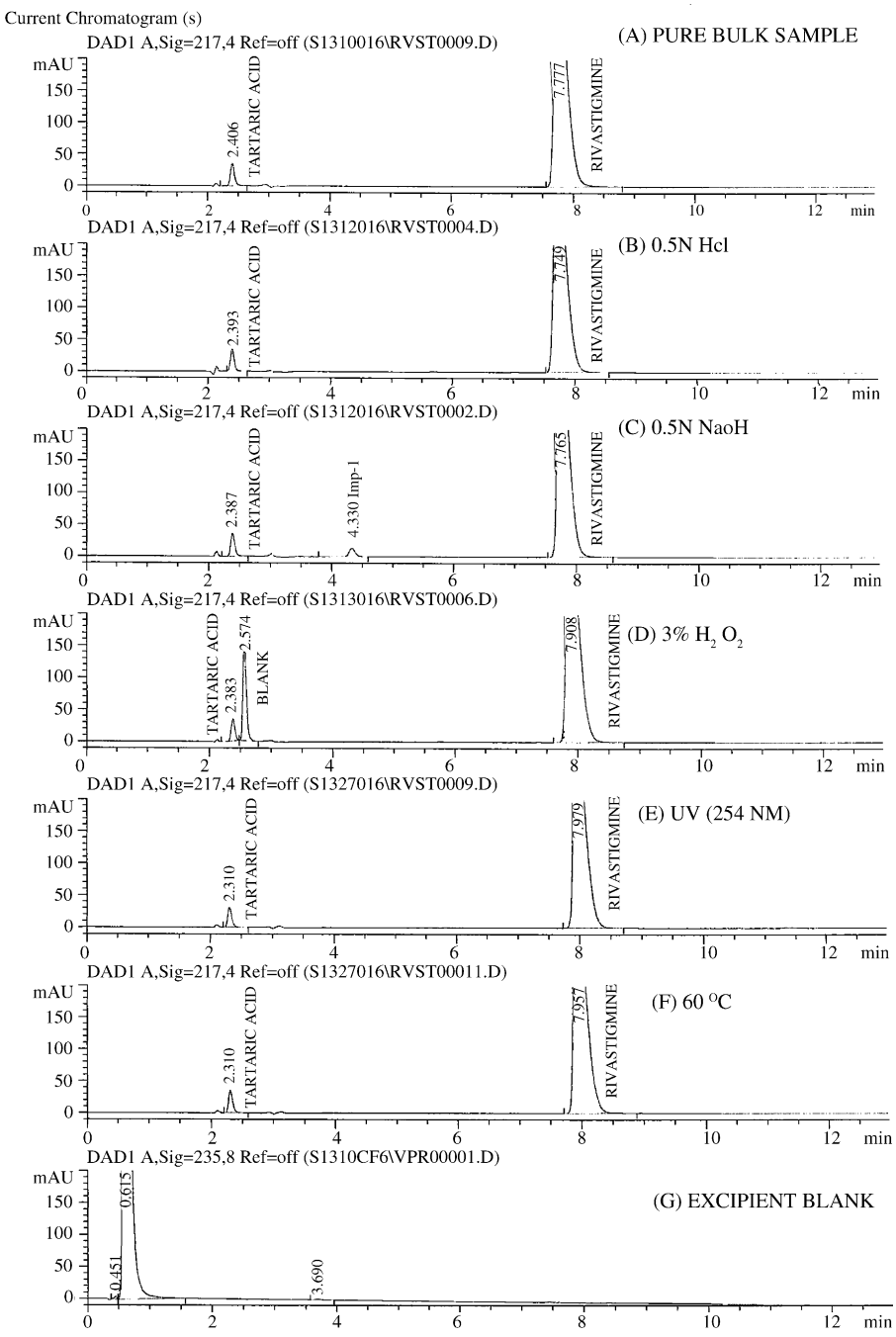


Fig. 2. Typical HPLC chromatograms of Rivastigmine hydrogen tartrate under stress conditions (A) pure bulk sample, (B) 0.5N HCl, (C) 0.5N NaOH, (D) 3% $H_2O_2$, (E) UV (254 nm), (F) 60 °C, and (G) excipient blank.

Rivastigmine hydrogen tartrate; the corresponding linear regression equation was $y = 25000x + 300$ with correlation coefficient greater than 0.999. Linearity was checked for assay method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 1.2 and 6, respectively. The results show that an excellent correlation existed between the peak area and concentration of Rivastigmine hydrogen tartrate.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ (0.06%) to 0.3% for Imp 1; the corresponding linear regression equation was $y = 4550x + 50$ with correlation coefficient greater than 0.998. Linearity was checked for related substance method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 3.5 and 12, respectively. The results show that an excellent correlation

Table 3
Recovery results of Rivastigmine hydrogen tartrate in bulk drug sample

| Added (μg) ($n = 3$) | Recovered (μg) | Recovery (%) | R.S.D. (%) |
|---|---|---|---|
| 52 | 51.6 | 99.2 | 0.7 |
| 104 | 104.8 | 100.8 | 0.3 |
| 156 | 158.0 | 101.3 | 0.9 |

$n = 3$ determinations.

Table 4
Recovery results of Rivastigmine hydrogen tartrate in formulation sample

| Added (μg) ($n = 3$) | Recovered (μg) | Recovery (%) | R.S.D. (%) |
|---|---|---|---|
| 49 | 49.1 | 100.2 | 0.4 |
| 98 | 96.6 | 98.6 | 0.6 |
| 147 | 149.2 | 101.5 | 0.7 |

$n = 3$ determinations.

existed between the peak area and concentration of Imp 1.

### 3.6. Accuracy

The percentage recovery of Rivastigmine hydrogen tartrate in bulk drug samples was ranged from 99.2 to 101.3 (Table 3). The percentage recovery of Rivastigmine hydrogen tartrate in formulation samples was ranged from 98.6 to 101.5 (Table 4). The percentage recovery of Imp 1 in bulk drugs samples was ranged from 95.2 to 104.3. HPLC chromatograms of unspiked and spiked Imp1 at 0.15% level in Rivastigmine bulk drug sample are shown in Fig. 3.

### 3.7. Robustness

In all the deliberate varied chromatographic conditions (flow rate, percentage organic strength, column temperature), the resolution between Rivastigmine and Imp1 was greater than four, illustrating the robustness of the method.

### 3.8. Solution stability and mobile phase stability

The % R.S.D. of assay of Rivastigmine during solution stability and mobile phase stability experiments was within 1% R.S.D. No significant change was observed in the content of Imp 1 during solution stability and mobile phase experiments. The solution stability and mobile phase stability experiments data confirm that Rivastigmine hydrogen tartrate

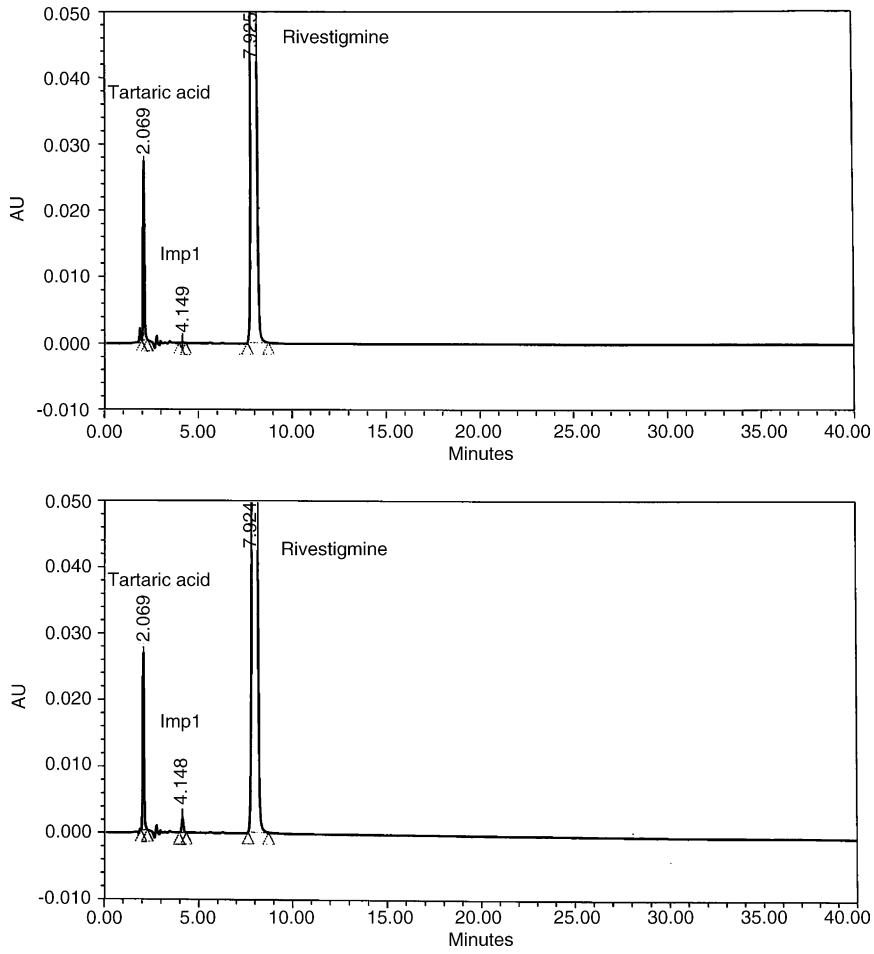


Fig. 3. HPLC chromatograms of unspiked and spiked Imp1at 0.15% level in Rivastigmine hydrogen tartrate bulk sample.

sample solutions and mobile phase used during assay and related substance determination were stable for at least 48 h.

## 4. Conclusions

The RPLC method developed for quantitative and related substance determination of Rivastigmine hydrogen tartrate is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for assessing the stability of Rivastigmine hydrogen tartrate as bulk drug. The developed method can be conveniently used for the assay determination of Rivastigmine hydrogen tartrate in formulations.

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