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# Identification, characterization and quantification of new impurities by LC–ESI/MS/MS and LC–UV methods in rivastigmine tartrate active pharmaceutical ingredient

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ABSTRACT

Six impurities were detected at trace level in rivastigmine tartrate drug substance by a newly developed high performance liquid chromatography method. Three impurities were characterized rapidly and three impurities were found to be unknown. The unknown impurities were enriched and identified with a combination of semi-preparative HPLC and LC/MS/MS techniques. Proposed structures were further confirmed by characterization using NMR, FT-IR, and EA techniques of impurity standards. Based on the spectroscopic, spectrometric and elemental analysis data unknown impurities were characterized as 3-[1-(dimethylamino)ethyl]phenyl *N*-ethyl-*N*-methyl carbamate *N*-oxide, ethyl-methyl-carbamic acid 4-(1-dimethylamino-ethyl)-phenyl ester and ethyl-methyl-carbamic acid 2-(1-dimethylamino-ethyl)-phenyl ester. A plausible mechanism for the formation of these impurities is also proposed. The method was validated according to ICH guidelines for fourteen impurities to demonstrate specificity, precision, linearity, accuracy and stability indicating nature of the method. Regression analysis showed correlation coefficient value greater than 0.999 for rivastigmine tartrate and its impurities. Accuracy of the method was established based on the recovery obtained between 93.41 and 113.33% for all impurities.

## 1. Introduction

Rivastigmine tartrate (Fig. 1a) is chemically (*S*)-3-[(1-dimethylamino)ethyl]phenyl *N*-ethyl-*N*-methyl carbamate hydrogen tartrate, which was synthesized as a single enantiomer because (*S*)-enantiomer is pharmacologically more potent than (*R*)-enantiomer [1]. It is a carbamate type reversible acetyl cholinesterase inhibitor used for symptomatic treatment of mild to moderate dementia in Alzheimer's disease and idiopathic Parkinson's disease. Alzheimer's disease is a progressive, irreversible brain disorder with no known cause or cure. It attacks the brain and slowly leads to memory loss, confusion, impaired judgment, personality changes, disorientation and loss of language skills. It is estimated that there are currently about 35 million people worldwide affected by Alzheimer's disease and is expected to triple by 2025 [2,3].

Several methods that have been reported in the literature for rivastigmine tartrate, some of which include a stability indicating LC method [4], voltammetric behavior [5], a validated chiral LC method for the enantiomeric separation [6], identification and characterization of new impurities [7], stability indicating HPTLC determination in the bulk drug and in pharmaceutical dosage forms [8] and few LC–MS methods for the analysis of rivastigmine tartrate in biological samples [9,10]. Recently HPLC methods for the determination of related substances have also been reported in US pharmacopoeia and pharmaeuropa [11,12].

ICH guidelines indicate that unknown impurities at or above 0.05% in the drug substance require identification [13] depending on the maximum daily dosage. Presence of impurities in drug substance can have significant impact on the quality, safety and efficacy of drug products. As a common practice, efforts should be made to identify and characterize all unknown impurities in the drug substance due to the ever increasing demand from regulatory agencies to manufacture high purity drug substances. Impurity profiling of drugs is almost the most important issue in the modern pharmaceutical analysis [14–20] for developing process technology to manufacture high purity drug substance. In recent years liquid chromatography coupled with mass spectrometry (LC/MS/MS) has emerged as an essential and versatile tool for structural elucidation of impurities [21,22].

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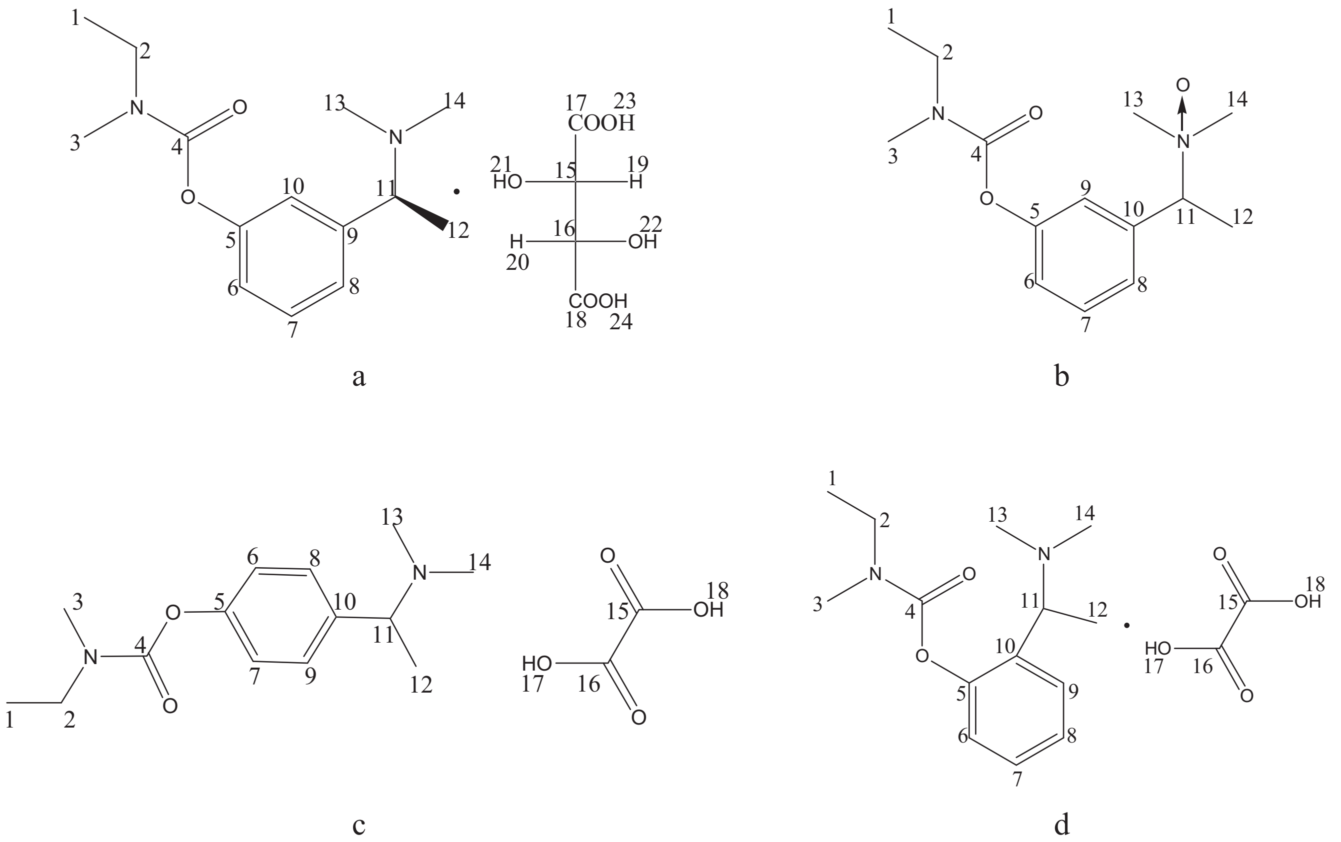

**Fig. 1.** (a) Rivastigmine tartrate, (b) Imp-A, (c) Imp-B, (d) Imp-C. *Numbering has been assigned only for NMR characterization.

A critical evaluation of HPLC methods [4,7,11,12] revealed that specificity and resolution of all known impurities (Table 1) were a major concern which were essential requirements for quantitative determination of impurities. Further use of very high concentration of ion pair reagent in mobile phase significantly reduces the column life resulting in expensive routine analysis. Therefore, it was strongly felt necessary to develop simple, efficient, sensitive, selective and cost effective RP-HPLC method. It should be noted that selective and sensitive analytical methods are required for quantitative analysis of impurities in drug substance.

The objective of the current study was to identify and characterize six impurities detected in low level during a HPLC investigation of rivastigmine tartrate lab sample. Three impurities were found to be reported previously [9,11] and three impurities were unknown. Impurities were enriched by semi-preparative HPLC and identified by liquid chromatography–tandem mass spectrometry using an electrospray ionization source and Q-trap mass analyser. The structure of the unknown impurities in rivastigmine tartrate was additionally confirmed by characterization using impurity reference standards with the help of NMR, IR, and EA techniques. This paper describes in detail about method development, identification and characterization of unknown impurities, degradation study and identification of degradants. Method was validated as per ICH guidelines [24]. A plausible mechanism for the formation of new impurities was proposed. To the best of our knowledge, these impurities have not been reported previously.

## 2. Experimental

### 2.1. Materials and reagents

Sample of rivastigmine tartrate API (batch No. RVS-crude) and standards of Imp-A, Imp-B, Imp-C, Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10 and Imp-11 were obtained from Chemical Research and Development Department, Jubilant Life Sciences Limited (Noida, India). Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). HPLC grade methanol, acetonitrile, dipotasssium hydrogen phosphate, ammonium formate, ammonium bicarbonate, analytical reagent grade ammonia solution and ortho phosphoric acid were purchased from Merck India Limited (Mumbai, India). Dimethyl sulphoxide-d6 (for NMR) was purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Potassium bromide FT-IR grade was purchased from Merck KGaA (Darmstadt, Germany).

### 2.2. High performance liquid chromatography

Samples were analysed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters Corporation, Milford, MA, USA) using an Xterra RP-18, (250 mm × 4.6 mm, 5 $\mu$m, Waters Corporation, Milford, MA, USA). A mobile phase consisted of A, 10 mM dipotassium hydrogen phosphate adjusted to pH 7.6 ± 0.05 with orthophosphoric acid–acetonitrile (90:10, v/v) and B, acetonitrile–methanol (60:40, v/v) with a timed gradient mode *T* (min)/%B: 0/5, 3/10, 10/20, 20/35, 55/55 60/5 70/5. A flow rate of 1.0 mL/min was used throughout the analysis. The injection volume was 10 $\mu$L for a sample concentration of 0.5 mg/mL prepared in diluent (mobile phase A-methanol, 40:60, v/v). Detector wavelength was fixed at 210 nm and the column temperature was maintained at 40 °C.

### 2.3. Semi-preparative HPLC

The impurities were enriched from rivastigmine tartrate lab sample, using a Shimadzu semi-preparative HPLC system consisted