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# Determination of serum amantadine by liquid chromatography-tandem mass spectrometry

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**Abstract**

*Background:* Amantadine (1-adamantylamine) is used for treatment of influenza, hepatitis C, parkinsonism, and multiple sclerosis. Current amantadine analysis by HPLC or gas chromatography (GC) requires a laborious sample pretreatment with extraction and/or derivatization steps. We established an LC-MS/MS method without protein precipitation, centrifugation, extraction and derivatization steps.
*Material and methods:* 50 μl sample + 50 μl of 0.4 mg/l 1-(1-adamantyl)pyridinium bromide as internal standard + 1000 μl water (96-well plate). Of this 25 μl + 500 μl water (96-well plate; final serum dilution 1:462). LC-MS/MS: Surveyor MS pump, Autosampler, triple-quadrupole TSQ Quantum mass spectrometer (Thermo Electron). Autosampling: 2 μl of each sample. Chromatography: isocratic water/acetonitrile (60/40 v/v) with 5 g/l formic acid, flow rate 0.2 ml/min, run time 3 min, Phenomenex Luna C8(2) (100 × 2.0 mm (i.d.); 3-μm bead size) column. Mass spectrometry: electrospray atmospheric pressure ionization, positive ion and selective reaction monitoring mode, ion transitions *m*/*z* 152.0→135.1 (at 22 eV amantadine) and 214.1→135.1 (at 26 eV internal standard).
*Results:* Calibration curves were constructed with spiked serum samples (amantadine 50–1000 μg/l, $r > 0.99$). No carry over (5000 μg/l). No ion suppression with retention times similar to those of amantadine (1.8 min) and the internal standard (2.1 min). Detection limit 20 mg/l, linearity 20–5000 mg/l, intra-assay/inter-assay CV < 6%/< 8%, recovery 99–101%. Method comparison: LC-MS/MS = 1.23 × GC-45 (Passing-Bablok regression). No significant bias between GC and LC-MS/MS (Bland-Altman plot).
*Conclusion:* We consider the sample pretreatment without deproteination, derivatization and centrifugation steps and the specificity of the tandem mass spectrometry as the most important points of our amantadine analysis method.
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*Keywords:* Adamantylamine; Amantadine; Liquid chromatography-tandem mass spectrometry; LC-MS/MS; Serum; Therapeutic drug monitoring; Automation

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## 1. Introduction

Amantadine (1-Adamantylamine) has been used for many years as an antiviral drug [1,2]. A computer-assisted literature search (Knowledge Finder) for "amantadine" yielded 200 hits for 2003–2004. Amantadine is used in combinations with others for the treatment of influenza A [2,3], hepatitis C [4,5], parkinsonism [6,7], and multiple sclerosis [8,9]; however, this can cause drug interactions with positive and adverse effects regarding patient outcome. Thus, amantadine improved the viral suppression by interferone and ribavirin in hepatitis C patients [5], showed a potentiated antidyskinetic action in combination with levetirazetam in patients with Parkinson's disease [10] or caused intense and recurrent paramnesic symptoms (deja vu experiences) under concomitant amantadine–phenylpropanolamine treatment of influenza [11]. Side effects of amantadine, like nausea, dizziness and insomnia are the most frequent in patients (5%–15%) receiving amantadine for 6 weeks [2]. The major side effects are almost amphetamine-like, including jitteriness, anxiety, nightmares, and occasionally hallucinations [2]. A blood amantadine concentration exceeding 1600 μg/l is considered toxic [5]. High dose amantadine treatment caused serious adverse events like myocardial infarction and a suicide attempt; others included impotence, confusion, alopecia, and hoarseness [5]. Drug interactions and side effects should be dependent on the serum amantadine concentration. Assessment of these effects and testing the patient's compliance requires a reliable analysis. Current analysis of serum amantadine requires a laborious sample pretreatment with organic solvent extraction (often 2–3 extraction and re-extraction cycles) and/or a derivatization followed by gas chromatography (e.g. [12,13]), HPLC [14,15] or gas chromatography/mass spectrometry [16,17]. This might be one reason why routine amantadine analysis is offered by only a few laboratories and why therapeutic amantadine monitoring is not common. Tandem mass spectrometry can significantly reduce sample pretreatment, workload, and analysis costs and improves specificity and sample throughput [18]. We established a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method for amantadine analysis in human serum. We aimed at a 96-well plate format for sample pretreatment and autosampling without protein precipitation, centrifugation, extraction or derivatization steps.

## 2. Material and methods

### 2.1. Reagents

The following chemicals and reagents were used: 1-adamantylamine-HCl (99+%) and 1-(1-adamantyl)pyridinium bromide (98%) (Sigma-Aldrich, Taufkirchen Germany), acetonitrile grad. grade and methanol grad. grade (Merck, Darmstadt, Germany), formic acid p.a. mass spectrometry (Fluka, Taufkirchen, Germany). A Milli-Q® water purification system (Millipore, Schwalbach, Germany) was used to obtain deionized water for serum dilution and HPLC analysis. Certified blood bank serum was obtained from the University of Mainz (Mainz, Germany).

### 2.2. Preparation of stock solutions

The primary stock solution of 1-adamantylamine (1 mg/ml) was prepared by dissolving 124 mg 1-adamantylamine-HCl in 100 ml water (corresponding to 100 mg free 1-adamantylamine/100 ml or 1 mg/ml). The working solution of 1-adamantylamine (40 μg/ml) was obtained by diluting 1.0 ml of the primary stock solution with water to a final volume of 25 ml. The primary stock solution of the internal standard (250 μg/ml) was prepared by dissolving 2.5 mg 1-(1-adamantyl)pyridinium bromide in 10 ml methanol. The working solution of the internal standard (0.5 μg/ml) was obtained by diluting 200 μl of the stock solution with water to a final volume of 100 ml. Stock and working solutions were aliquoted and stored at $-20$ °C for 6 months.

### 2.3. Calibration curves

Blood bank serum was spiked with 1-adamantylamine working solution to give the following concentrations: 50 μg/l (12.5 μl working solution in 10 ml serum), 100 μg/l (25 μl in 10 ml), 200 μg/l (125 μl in 10 ml), and 1000 μg/l (250 μl in 10 ml). Aliquots of these solutions were stored at $-20$ °C for 12 months. These four calibrators were used for calibration in each routine analysis run.

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