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# Application of a novel liquid chromatography/tandem mass spectrometry method for the determination of antazoline in human plasma: Result of ELEPHANT-I [ELEctrophysiological, pharmacokinetic and hemodynamic effects of PHenazolinum (ANTazoline mesylate)] human pharmacokinetic study

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ABSTRACT

Antazoline is a first-generation antihistaminic agent with antiarrhythmic quinidine-like properties. In some countries, it is widely used for termination of cardiac arrhythmias, especially atrial fibrillation (AF). However, no human pharmacokinetic studies have been conducted with intravenous antazoline. The aim of our study was to develop and validate a novel liquid chromatography/tandem mass spectrometry (LC–MS/MS) method for the determination of antazoline in human plasma: the ELEPHANT-I [ELEctrophysiological, pharmacokinetic and hemodynamic effects of PHenazolinum (ANTazoline mesylate)] human pharmacokinetic study. Antazoline was extracted from plasma using liquid–liquid extraction. The concentration of the analyte was measured by LC–MS/MS with xylometazoline as an internal standard. The method was validated for linearity, precision, accuracy, stability (freeze/thaw stability, stability in autosampler, short and long term stability), dilution integrity and matrix effect. The analyzed validation criteria were fulfilled. The method was applied to a pharmacokinetic study involving 10 healthy volunteers. Following a single intravenous dose of antazoline mesylate (100 mg), the plasma concentration profile showed a relative fast elimination with a terminal elimination half-life of 2.29 h. A relatively high volume of distribution was observed ($V_{ss}$ = 315 L). The values of mean residence time ($MRT_\infty$), area under the curve ($AUC_\infty$) and clearance were 3.45 h, 0.91 mg h $L^{-1}$ and 80.5 L $h^{-1}$, respectively. One volunteer showed significant differences in pharmacokinetic parameters. In conclusion, the proposed new LC–MS/MS method was successfully used for the first time for the determination of antazoline in human plasma.



## 1. Introduction

Antazoline is an ethylenediamine derivative and a first-generation antihistamine that exhibits antihistamine, anticholinergic, and central nervous system inhibitory effects. It is most commonly used to relieve the local symptoms of allergic conjunctivitis, for nasal congestion, and in eye drops. It is occasionally administered intravenously to relieve the symptoms of an immediate allergic reaction [1].

In addition, antazoline is an antiarrhythmic agent with quinidine-like properties. In the past, antazoline was classified as a sodium channel blocker (Ia class) according to the Vaughan-Williams classification. In some countries, it is registered and

*Abbreviations:* AF, atrial fibrillation; AN-PAF, Clinical efficacy of ANtazoline in rapid cardioversion of Paroxysmal Atrial Fibrillation: a single center, randomized, double blind, placebo-controlled study; $AUC_t$, area under the plasma concentration-time curve from time zero to time t; $AUC_\infty$, area under the plasma concentration-time curve from time zero to infinity; $C_0$, back-extrapolated plasma drug concentration at time zero following bolus intravenous injection; Cl, apparent total body clearance of the drug from plasma; D, dose; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; LLE, liquid–liquid extraction; $MRT_t$, mean residence time from time zero to time t; $MRT_\infty$, mean residence time from time zero extrapolated to infinity; QC, quality control sample; $t_{1/2}$, elimination half-life; $V_{ss}$, apparent volume of distribution at steady state.

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widely used for termination of cardiac arrhythmias, especially atrial fibrillation (AF). It is known from literature that antazoline is effective in termination of AF, but owing to the lack of large randomized trials, the drug is not listed in any of the formal guidelines [2–5]. Recently, the Antazoline in Rapid Cardioversion of Paroxysmal Atrial Fibrillation Study (the AnPAF Study)—the first randomized trial that evaluated the efficacy of antazoline for termination of AF, was completed and the results were announced at the European Society of Cardiology meeting held in London in 2015. This study demonstrated the high efficacy (72% vs. 10% of placebo) and safety of antazoline in termination of AF [6,7]. Furthermore, in another study performed on rabbit hearts, antazoline was more effective in suppressing AF than flecainide, a well-established and highly effective antiarrhythmic drug [8].

Although the drug has been used in clinical practice for a long time, pharmacokinetic studies have not been conducted. The only pharmacokinetic data available to date comes from studies performed in beagle dogs and rats. Moreover, the only method applied for the determination of antazoline concentration in plasma is the high-performance liquid chromatography HPLC-UV method [9,10]. HPLC with UV detection is a relatively inexpensive method having the disadvantages of low sensitivity and selectivity. Thus, some interferences from, e.g., other pharmaceuticals taken by patients, are possible [11]. Nowadays, LC–MS/MS (liquid chromatography coupled to tandem mass spectrometry) methods are widely used in clinical medicine and pharmacy owing to high selectivity and sensitivity [12].

Recently, interest in antazoline as an antiarrhythmic agent has increased. Since no studies have assessed the pharmacokinetic profile of antazoline in humans, the optimal dosing and mode of drug administration are yet to be established. Thus far, antazoline dosing has been based on clinical experience; hence, doses and infusion rates suggested by various authors are variable [3–6]. According to the Summary of Product Characteristics, antazoline mesylate should be administered in 100 mg bolus for a few minutes, up to a maximum dose of 300 mg. However, as highlighted previously, this recommendation is not based on any solid data since pharmacokinetics of antazoline is unknown [1].

The aim of our study was to develop and validate a novel LC–MS/MS method for the determination of antazoline in human plasma. The method was applied to a pharmacokinetic study of intravenous bolus of antazoline in healthy volunteers.

This study is a part of the ELEPHANT I study [ELEctrophysiological, pharmacokinetic and hemodynamic effects of PHenazolinum (ANTazoline mesylate)], which evaluates the pharmacokinetic profile of antazoline and its impact on hemodynamic, electrocardiographic, and electrophysiological parameters.

## 2. Materials and methods

### 2.1. Chemicals

The reference standard of antazoline mesylate was a kind gift from Polfa Warszawa S.A (Poland). Xylometazoline, oxymetazoline, and phenacetin (internal standards, IS) were purchased from Sigma-Aldrich (St. Louis, US). The solvents, HPLC gradient-grade methanol, acetonitrile, and formic acid 98% were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from the Millipore water purification system (Milli-Q, Billerica, US). Blank plasma was obtained from the Regional Center of Blood Donation and Treatment (Warsaw, Poland).

### 2.2. Chromatographic and mass spectrometric conditions

The instrumental analysis was performed using Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, US) equipped with a degasser, an autosampler, and a binary pump coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer QTRAP 4000 (AB Sciex, Framingham, MA, US). The Turbo Ion Spray source was operated in the positive mode. The curtain gas, ion source gas 1, ion source gas 2, and collision gas (all high purity nitrogen) were set at 345 kPa psi, 207 kPa, 276 kPa, and "high" instrument units, respectively. The ion spray voltage and source temperature were 5000 V and 600 °C, respectively. The target compounds were analyzed in the MRM mode (Table 1). The mass spectra for antazoline at collision energies 10–50 V are shown in Fig. A.1 .

Chromatographic separation was achieved with a Kinetex C18 column (100 mm × 4.6 mm, 5 μm, Phenomenex, Milford, US). The column was maintained at 25 °C at a flow rate of 0.75 mL $min^{-1}$. The mobile phases consisted of HPLC-grade water with 0.1% formic acid as the eluent A and methanol with 0.1% formic acid as the eluent B. The gradient (%B) was as follows: 0 min 20%; 1 min 20%; 3 min 95%; 9 min 95%. The re-equilibration of the column to the initial conditions lasted 2 min. The injection volume was 5 μL.

### 2.3. Standard solutions, calibration standards, and quality control samples

The standard stock solutions of antazoline and IS (1 mg $mL^{-1}$) were prepared by dissolving an appropriate amount of the substance in methanol. The working solutions (10 μg $mL^{-1}$) were prepared by diluting standard stock solutions with water. The solutions were stored at 4 °C. The calibration standards for antazoline were prepared on human plasma at concentrations: 5–3000 ng $mL^{-1}$. The quality control (QC) samples were prepared on human plasma at the following concentrations: 10, 1000, and 2500 ng $mL^{-1}$. The calibration standards and QC samples were stored at −80 °C until use. Sodium citrate was used as an anticoagulant.

### 2.4. Sample preparation

Plasma sample (0.1 mL) was mixed with the IS (20 μL) and 1 M NaOH (10 μL), and shaken for 5 min with ethyl acetate (1 mL). After centrifugation (10 min at 2000*g*), the organic layer was evaporated to dryness (40 °C). The residue was reconstituted in 75% aqueous methanol (400 μL), diluted 5 times with water, and 5 μL aliquot was injected into the LC–MS/MS. The method was validated according to the international guidelines. Each batch consisted of three levels of QC samples (10, 1000, and 2500 ng $mL^{-1}$) in order to validate the results.

### 2.5. Method validation

The validation was performed for this study according to the European Medicines Agency [13] and US Food and Drug Administration [14] guidelines. Briefly, the linearity range was selected as 5–3000 ng $mL^{-1}$. Ten points calibration curve was prepared in pentadruple. The accuracy and precision of the method were determined within run (n = 5) and between run (n = 15) using LLOQ (5 ng $mL^{-1}$) and QC samples (10, 1000, and 2500 ng $mL^{-1}$). Carry-over wherein blank human plasma samples were analyzed following the highest calibration standards was also studied. The matrix effect was calculated following the method by Matuszewski et al. [15]. The absolute matrix effect (ME) was obtained by comparison of the analyte peak areas in solvents spiked with standards and post-extraction spiked plasma samples. The relative matrix effect was calculated as a variability (RSD) of the ratios of the respective absolute matrix effects for the analyte and internal standard. Various lots of matrix used for the test included hemolyzed and hyperlipidemic plasma samples.

Stability of antazoline mesylate working solution (10 μg $mL^{-1}$) during analysis was determined via repeated analysis of samples