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Method development for quantification of the environmental neurotoxin annonacin in Rat plasma by UPLC–MS/MS and application to a pharmacokinetic study

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ABSTRACT

Annonacin is an environmental neurotoxin identified in the pulp of several fruits of the Annonaceae family (for example in *Annona muricata*, *Asimina triloba*), whose consumption was linked with the occurrence of sporadic atypical Parkinsonism with dementia. Pharmacokinetic parameters of this molecule are unknown. A method for its quantification in Rat plasma was developed, using its analogue annonacinone as an internal standard. Extraction from plasma was performed using ethylacetate with a good recovery. Quantification was performed by UPLC–MS/MS in SRM mode, based on the loss of the γ-methyl-γ-lactone (−112 amu) from the sodium-cationized species $[M+Na]^+$ of both annonacin and internal standard. The limit of quantification was 0.25 ng/mL. Despite strong matrix effects, a good linearity was obtained over two distinct ranges 0.25–10 ng/mL and 10–100 ng/mL. The intra- and inter-day precisions (RSD) were lower than 10%, while accuracy was within ±10%. This method was applied to a pharmacokinetic study in the Rat. After oral administration of 10 mg/kg annonacin, a C_{max} of 7.9 ± 1.5 ng/mL was reached at T_{max} 0.25 h; $T_{1/2}$ was 4.8±0.7 h and apparent distribution volume was 387.9 ± 64.6 L. The bioavailability of annonacin was estimated to be 3.2 ± 0.3% of the ingested dose.

1. Introduction

Annonaceous acetogenins (AAGs) constitute a class of lipophilic polyketides specifically distributed in the Annonaceae plant family [1]. These compounds have been extensively studied as antitumoral drug-candidates [2,3]. More recently, AAGs have been proposed as dietary neurotoxins [4,5], possibly responsible for clusters of Parkinsonism/dementia in tropical populations, as evidenced by epidemiological studies conducted in the French West Indies [6]. Consistent with this hypothesis, AAGs are potent inhibitors of mitochondrial complex I [1,5] and were evidenced to be neurotoxic in vivo, in rodent models of neurodegeneration and of tauopathies, after intravenous or subcutaneous sub-chronic administration [7,8].

AAGs were identified in several pantropical fruits such as soursop (*Annona muricata*) [9]. Recent methodological development allowed their detection in other Annonaceae-derived food products [10,11], revealing alimentary exposure to constitute a possibly wide public health problem. The most abundant AAG in soursop is annonacin [10,12], which was also evidenced in high amounts in the fruit pulp of the north-American pawpaw (*Asimina triloba*) [13,14]. Hence it has become a major concern to evaluate the pharmacokinetic parameters of this molecule, as the chef-de-file of AAGs, as part of sanitary risk evaluation [15]. Pharmacological study of an Annonaceae extract suggested intestinal absorption of these

Abbreviations: AAG, Annonaceous acetogenin; amu, atomic mass unit; AUC, area under curve; Cl, clearance; EMA, Eropean Medicines Agency; F_{obs} apparent bioavailability; IS, internal standard; LLOQ, lower limit of quantification; MF, matrix factor; MQC, medium concentration quality control; QC, quality control; ULOQ, upper limit of quantification; RSD, relative standard deviation; SRM, selected reaction monitoring; $T_{1/2}$, half-life time; T_{max}, time of maximum concentration; UPLC–MS/MS, ultra performance liquid chromatography tandem mass spectrometry; Vz, distribution volume.

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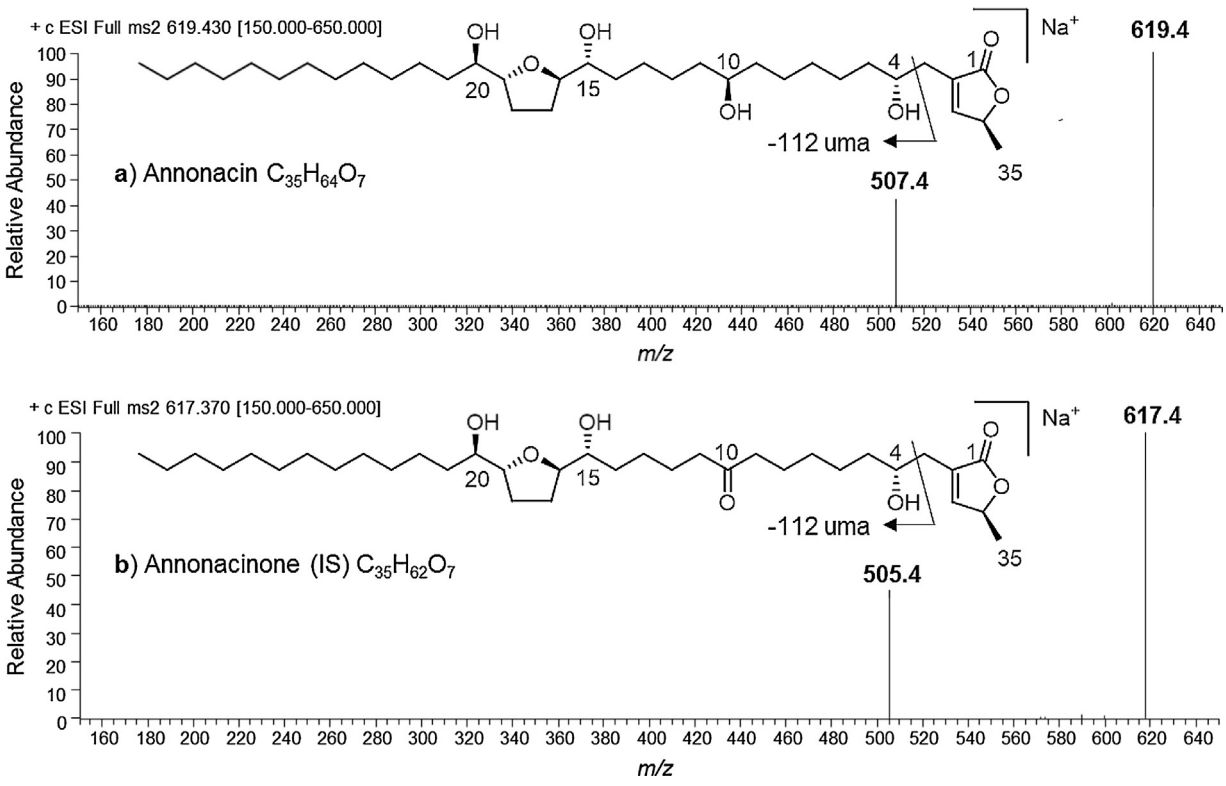

Fig. 1. Production mass spectra of $[M+Na]^+$ adducts of (**a**) annonacin and (**b**) annonacinone (IS).

compounds, with the observation of an antitumoral activity in mice after oral-route administration [16]. Nevertheless, Dang et al. evidenced the possible uptake of AAGs by intestinal epithelium in an ex-vivo Rat model [17]. Although a method for the quantification of the AAG bullatacin (rollinastatin-2) in Rat plasma by single ion monitoring HPLC-ESI–MS was published [18], no data about the bioavailability of any AAG is available, to our knowledge. In this context, we developed a method for the extraction and quantification of annonacin in Rat plasma. This process was validated according to EMA guidelines [19], and then applied to a preliminary pharmacokinetic study in the Rat.

2. Experimental

2.1. Chemical and reagents

Annonacin and annonacinone (internal standard, IS) (purity > 97%) were purified from the seeds of *Annona muricata* L., as previously described [1]. Ethylacetate, ethanol and DMSO were supplied by Carlo Erba SDS (Val de Reuil, France). Isopropanol (HPLC-grade) was supplied by Fischer Scientific SAS (Illkirch, France). Methanol and acetonitrile (HPLC-grade) were supplied by JT Baker (Paris, France). Ultra-pure water was prepared in-house with a Millipore Milli-Q purification system (Darmstad, Germany). Olive oil (European Pharmacopoeia-compliant) was supplied by Cooper (Melun, France). Wistar Rat plasma was purchased from Janvier (St Quentin-Fallavier, France).

2.2. UPLC–MS/MS method

Analyses were performed with a Dionex Ultimate 3000 RSLC system. Successful chromatographic separation between annonacin and IS was obtained at 40 °C with an Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm, Waters, Guyancourt, France) attached to an Acquity UPLC BEH C_{18} VanGuard pre-column (2.1 × 5 mm, 1.7 μm), with an isocratic mobile phase consisting of water-acetonitrile 35:65 during 1 min, then a water-acetonitrile gradient from 35:65–15:85 in 5 min, at a flow rate of 0.5 mL/min. A 2 min isopropanol-acetonitrile 50:50 washout was applied after each run.

Mass spectra were recorded with a Triple quadrupole TSQ Vantage EMR (Thermo Scientific, Les Ulis, France), equipped with a heated ESI source in the positive ion mode. The source parameters were set as follows: spray voltage 2000 V, vaporizer temperature 350 °C, sheath gas (N_2) pressure 45 psi, auxiliary gas pressure (N_2) 20 psi, capillary temperature 300 °C. Analysis was performed under the Selected Reaction Monitoring (SRM) mode using the transitions *m/z* 619.4 g 507.4 for annonacin and *m/z* 617.4 g 505.4 for the IS, with collision energies set at 36 eV and 37 eV for annonacin and for the IS, respectively. Data acquisition was performed using Thermo Scientific Xcalibur 2.1 software system.

2.3. Preparation of calibration standards and quality control

Stock solutions of annonacin and IS were prepared at a concentration of 1 mg/mL in methanol and stored at −20 °C. Working standard solutions of annonacin ranging from 1 ng/mL to 1 μg/mL were prepared daily by successive dilutions of stock solution in methanol. Stock solution of IS was diluted daily to prepare a working solution at a concentration of 100 ng/mL.

Calibration standards were prepared by spiking 100 μL of pooled plasma with 10 μL of IS working solution to reach the concentration 10 ng/mL and with 10 μL of annonacin in solution of appropriate concentration, to reach concentrations of 0.25; 0.5; 0.75; 1; 2.5; 5; 7.5; 10; 15; 20; 25; 50; 75; 100 ng/mL, respectively. In order to evaluate intra- and inter-day precisions and accuracy, Quality Control (QC) samples were prepared in plasma at concentrations 0.25; 0.75; 2.5; 10; 100 ng/mL.