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LC–ESI-MS/MS characterization of strophanthin-K

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Abstract

A liquid chromatography–mass spectrometry (LC–MS) method was developed for the characterization of strophanthin-K, a mixture of cardiac glycosides extracted from the seeds of *Strophanthus kombè*. The method is based on the separation of the cardenolides using high performance liquid chromatography (HPLC) followed by detection with electrospray ionization mass-spectrometry (ESI-MS). Chromatographic separation of the analytes was achieved on a RP C-18 column using water: 1% formic acid in water (v/v):acetonitrile gradient mobile phase. Strophanthin-K glycosides studied in ESI-MS in negative ion mode formed abundant adduct ions $[M + HCOO]^-$ while the pseudomolecular ions $[M - H]^-$ are obtained in ESI-MS/MS experiments. Six different cardiac glycosides were identified and characterized: k-strophanthoside, k-strophanthin-β, helveticoside (erysimin), erysimoside, cymarin and neoglucoerysimoside.

Forced degradation investigations done with strophanthin-K showed that k-strophanthidin (the aglycone of strophanthin-K glycosides) was the main product of degradation in acidic conditions; however, in basic conditions, the hydrolysis of the unsaturated 17β-lactones to the corresponding γ-hydroxy acids was the predominant degradation pathway.

Keywords: Strophanthin-K; k-Strophanthoside; Cardiac glycosides; Cardenolides; LC–ESI-MS/MS

1. Introduction

The cardiac glycosides are a class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Since the desirable cardiotonic action is of particular benefit in the treatment of congestive heart failure and associated oedema, cardiac glycosides represent one of the most important drug classes available.

Among these ones, the mixture of glycosides extracted from the seeds of *Strophantus kombé*, a climbing African plant belonging to the Apocynaceae family, is called strophanthin-K [1].

The therapeutic effects of all cardiac glycosides on the heart are qualitatively similar, but differ strongly in their pharmacokinetic properties, which mainly reflect their polarity. In particular, strophanthin-K is characterized by a short lasting-effect and by a greater diuretic power, which is esteemed of value in cases complicated by oedema [2].

The composition of the mixture was found to vary, k-strophanthoside being the major component. Other glycosides (Fig. 1), containing the same aglycone strophanthidin but with different sugars moiety, were found in strophanthin-K: cymarin, k-strophanthin-β, erysimoside, helveticoside and glucoerysimoside [3]. A little confusion was still present around the structure of this last glycoside as other authors [4] described instead the occurrence of neoglucoerysimoside in *Strophantus kombè* extract.

Cardenolides are also thermally labile and polar compounds: these features prevented their characterization by traditional electron-impact mass spectrometry. The development of more soft-ionization techniques allowed the detection and the characterization of this class of compounds. Indeed digoxin, digitoxin and other related glycosides were studied by FAB-MS [5] and also determined in biological fluids by hyphenated techniques as HPLC–ionspray mass

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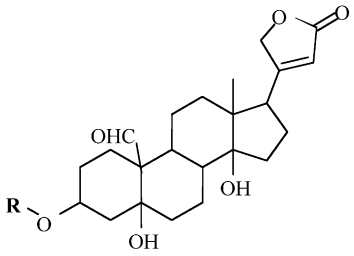

Compound	R	MW	Name	BF
1	Cym-Gluc-Gluc	872	K-Strophanthoside	$C_{42}H_{64}O_{19}$
2	Cym	548	Cymarin	$C_{30}H_{44}O_{9}$
3	Digit	534	Helveticoside	$C_{29}H_{42}O_{9}$
4	H	404	Strophanthidin	$C_{23}H_{32}O_{6}$
5	Digit-Gluc	696	Erysimoside	$C_{35}H_{52}O_{14}$
6	Cym-Gluc	710	K-Strophanthin-β	$C_{36}H_{54}O_{14}$
7	Digit-Gluc-Gluc	858	Neoglucoerysimoside	$C_{41}H_{62}O_{19}$

Fig. 1. Structures of the aglycone and glycosides from *Strophantus kombé* extract (strophanthin-K). Cym: cymarose, Gluc: glucose, Digit: digitoxose.

spectrometry [6] and HPLC–ESI-MS/MS [7]. However, mass spectrometric characterization of strophanthin-K glycosides was not so far reported in literature. Moreover, even if strophanthin-K has been described in a monography of the Italian Pharmacopoeia 9th edition [8], a certified reference standard was not available leading some difficulties in characterizing the composition of the different batches of production of extracts and of the potential degradation products.

In this report, an LC–ESI-MS/MS in negative ion mode method has been developed for the characterization of strophanthin-K glycosides as well as the degradation products of its main components obtained under stressed conditions.

2. Experimental

2.1. Chemicals

Methanol, acetonitrile (all HPLC grade), formic acid (50%, LC–MS grade), k-strophanthoside, helveticoside (erysimin), cymarin, were purchased from Sigma-Aldrich (Milano-Italy); strophanthidin was from ICN (Milano-Italy).

Sample of strophanthin-K was obtained from Pharmafar s.r.l. (Torino-Italy). Water was purified by Milli-Q instrument (Millipore Corp., Bedford, MA, USA).

2.2. Electrospray mass spectrometry (ESI-MS)

The ESI-MS spectra were acquired in negative ion mode using a Thermo Finnigan LCQ Deca XP *plus* Ion Trap Mass Spectrometer instrument from Thermo Finnigan (San Josè, CA, USA) equipped with an electrospray ion source (ESI) and an Xcalibur® system manager data acquisition software. Sample solutions (5 μg ml^{-1}) were infused in the ESI source using a syringe pump at a flow rate of 2 μl ml^{-1} and the mass scan range was *m/z* 100–1000. Operating conditions on the ion trap mass spectrometer in negative polarity were as follows: spray voltage, 5.0 kV; source current, 80 μA; capillary temperature, 250 °C; capillary voltage, −45 V; tube lens offset, −60 V; multipole 1 offset, 6 V; multipole 2 offset, 10 V; sheath gas flow (N_2), 30 A.U. Data were acquired in MS, MS/MS and MS^n scanning mode and for all MS/MS and MS^n experiments the precursor isolation window was set at 1 atomic mass unit (a.m.u.) and the collision energy was optimized at 27–33%.

2.3. Liquid chromatography/electrospray mass spectrometry (LC–ESI-MS)

The extract was analyzed by LC–ESI-MS "on-line" using the same instrument described above connected to a Surveyor HPLC system (Thermo Finnigan, San Josè, CA, USA) equipped with a quaternary pump, a Surveyor AS autosampler and a vacuum degasser. The chromatographic separation was performed on a Symmetry Shield C-18 column (150 mm × 4.6 mm i.d., with particle size of 5 μm) (Waters Corporation, Milford, MA, USA) maintained at 35 °C. The solvents used as mobile phase were A: water, B: 1% formic acid in water (v/v) and C: acetonitrile. At the start of analysis, the composition was 76:1:23 (v/v/v). After 12-min hold in these conditions, elution was performed by a linear gradient from 76:1:23 to 60:1:39 in 8 min and then maintained for 5 min at a constant flow rate of 0.8 ml min^{-1}; the sample injection volume was 20 μl.

The eluate was injected into the electrospray ion source with a splitting of 20% and the MS and MS/MS spectra were acquired and interpreted using the software Xcalibur®.