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Short communication

Hydrolysis and transesterification reactions of candesartan cilexetil observed during the solid phase extraction procedure☆

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Abstract

Candesartan cilexetil is an angiotensin receptor antagonist widely used in the treatment of high blood pressure. This prodrug is metabolised into candesartan, which blocks the receptors AT1 for angiotensin II decreasing the blood pressure levels. During the development of a solid phase extraction procedure for the chromatographic determination of eight antihypertensive compounds, lack of linearity and reproducibility was observed only for candesartan cilexetil. Due to this fact, a stability study for this prodrug was performed. It showed that the lack of linearity and reproducibility was based on hydrolysis and transesterification processes which occurred during the drying step after elution with methanol into glass tubes. These phenomena could be reproduced artificially under basic conditions, which demonstrated the presence of basic residues in glass tubes. The study of this potential hydrolysis and transesterification reactions is very important to assure that labile drugs containing ester groups remain unaffected.

Keywords: Candesartan cilexetil; Hydrolysis; Transesterification; Stability

1. Introduction

Candesartan cilexetil is an angiotensin II receptor antagonist (ARA-II) widely used in the treatment of high blood pressure. This prodrug is metabolised into candesartan (the carboxylic acid) by the action of some endogenous esterases [1] and the released drug blocks the receptors AT1 for angiotensin II decreasing the blood pressure levels.

The development of analytical methods for biological matrices is a complex procedure which involves extraction techniques prior to the separation step [2,3]. These techniques result in cleaner extracts, which increase the life-time of the columns and the chromatographic system, and reduce the appearance of interferences and ion suppression phenomena [4,5].

During the sample preparation steps, the studied compounds can suffer degradation and hydrolysis due to the exposure to heat, solvents, or the interaction between these agents and the materials that are employed, such as glass walls.

Some methods have been published in bibliography for the determination of candesartan cilexetil in biological samples. In all cases, extraction procedures are described, which involve solid phase extraction (SPE) [6] or liquid–liquid extraction (LLE) [7]. In both cases, glass tubes are usually employed, since glass is a material highly resistant to chemical reagents. Glass can be defined as a homogeneous material with a random, liquid-like (non-crystalline) molecular structure. The manufacturing process requires that the raw materials are heated to a temperature sufficient to produce a completely fused melt, which, when cooled rapidly, becomes rigid without crystallizing [8].

There are different glass types but the most frequently used in the laboratory are the borosilicate glass and the AR-Glas, which belongs to the soda lime group of glasses, with a high alkali and alkaline earth oxide content [9].

The AR-Glas is usually employed in the manufacturing of pipettes, vials and test tubes. The main chemical components of AR-Glas (expressed in approximate weight percent) are as

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follows: SiO_2 (69), B_2O_3 (1), K_2O (3), Al_2O_3 (4), Na_2O (13), BaO (2), CaO (5) and MgO (3) [9].

In this work, during the development of a new SPE-LC–MS/MS method for the determination of eight compounds of the ARA-II family, lack of linearity and repeatability for candesartan cilexetil was observed. The fact that the method was adequate for the rest of the studied compounds (valsartan, irbesartan, eprosartan, losartan and one of its metabolites, candesartan and telmisartan) indicated a problem of stability of candesartan cilexetil during the extraction procedure, which was not observed when standard solutions were injected into the chromatographic system.

This study demonstrates that candesartan cilexetil can undergo basic hydrolysis and transesterification reactions during the SPE procedure, which reduce the amount of this compound in the sample and increases the concentration of candesartan, which is the active drug and the product of the hydrolysis of candesartan cilexetil. In those cases in which a drug and its prodrug are present in the same sample, it is very important to check for these phenomena and develop strategies to avoid them, in order to obtain adequate precision and accuracy.

2. Experimental

2.1. Chemicals and reagents

Candesartan cilexetil [(±)-1-hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)l]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester)] and candesartan [2-ethoxy-1-((2′-(1*H*-tetrazol-5-yl)(1,1′-biphenyl)-4-yl)methyl)-1*H*-benzimidazole-7-carboxylic acid] were kindly supplied by AstraZeneca (Mölndal, Sweden). The structures for these molecules are shown in Fig. 1.

Reagent grade phosphoric acid and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Deionised water (<0.1 mS from a cartridge-deioniser, Memtech, Moorenweis, Germany), ammonium formate (Sigma, Deisenhofen, Germany), gradient grade acetonitrile and analytical grade formic acid (Merck, Germany) were used for HPLC solvents. Gradient grade methanol was purchased from Merck (Germany).

2.2. Instrumentation

The tested glass tubes and vials were purchased from different manufacturers. Five-millilitre AR-Glas tubes were obtained from Sarstedt (Nümbrecht, Germany), 1.5 mL plastic tubes from Eppendorf (Hamburg, Germany) and 1.5 mL vials for chromatography (transparent and brown vials) from Wicom (Heppenheim, Germany).

The SPE cartridges were Varian Bond Elut C8, 1 mL/100 mg (Varian, Harbour City, CA, USA).

The LC–DAD–MS/MS system consisted of an API 365 triple quadrupole mass spectrometer fitted with a turbo ionspray interface (Applied Biosystems/Sciex, Darmstadt, Germany), an Agilent 1100 series DAD (Agilent, Waldbronn, Germany) and a Shimadzu HPLC system (two pumps LC10AD Shimadzu, Duisburg, Germany). Analyses were performed with electrospray ionisation using a turbo ionspray source. A polar end-capped phenylpropyl reversed phase column (Synergy Polar-RP 150 mm × 2 mm, 4 μm) with a guard column (4 mm × 2 mm, same packing material) thermostated at 40 °C was used for the chromatographic separation (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of sol-

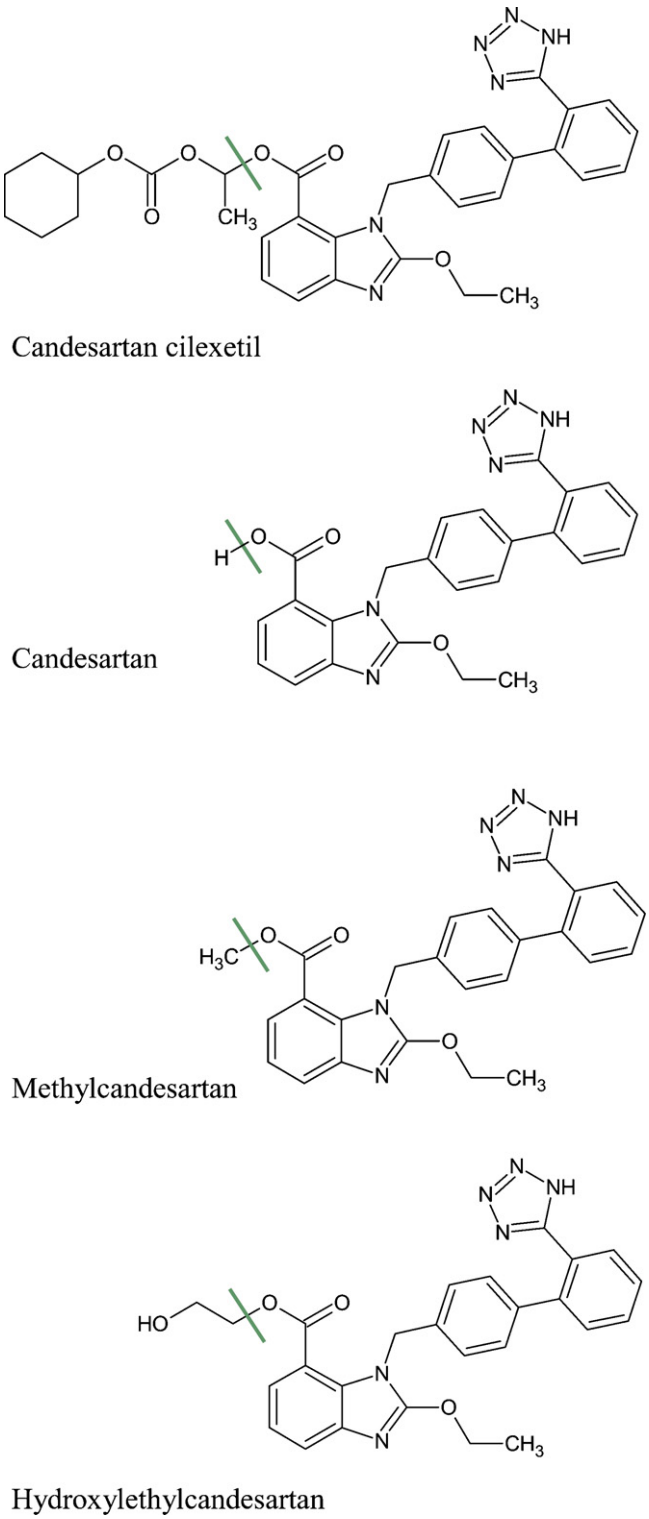

Fig. 1. Chemical structures of candesartan cilexetil and its derivates.

vent A (0.1% formic acid (v/v) with 1 mmol/L ammonium formate) and solvent B (acetonitrile: 0.1% formic acid 95:5 (v/v) with 1 mmol/L ammonium formate).

A 28 min gradient elution for UV spectra and single quadrupole mass spectra (Q1 scan) was applied at a flow rate of 0.25 mL/min (0–1 min: 5% B; 1–5 min: 5–30% B linear; 5–15 min 30–70% B linear; 15–19 min: 70–95% B linear; 19–22 min: 95% B; 22–24 min: 95-5% B linear; 24–28 min: 5% B).

The turbo ionspray source was operated at 350 °C with an ionisation voltage of 5250 V (positive mode), and nitrogen as curtain gas (11×10^{-5} Torr), nebulizer gas (10×10^{-5} Torr) and turbo gas (3 L/min). The Q1 ion scans were performed with the following MS parameters: three different defragmentation potentials (DP): 20, 50 and 80 V; focussing potential (FP): 200 V; entrance potential (EP): 7 V; and unit resolution for Q1. Step size was 0.1 amu.

2.3. Extraction procedure

The spiked human plasma samples (using methaqualone as internal standard) were extracted as follows: the SPE cartridges were conditioned with 2 mL of methanol, followed by 2 mL of phosphate buffer (50 mM, pH 2). The samples (1 mL of spiked plasma and 1 mL of phosphoric acid, centrifuged at 4000 rpm for 5 min) were applied to the cartridges manually and washed with 1 mL methanol/50 mM phosphate buffer, pH 2, 20/80 (v/v), followed by a 10 min drying period at high vacuum. The cartridges were then eluted with 2 mL of methanol. The eluate was evaporated to dryness under nitrogen at 60 °C. The residue was reconstituted with 100 μL of mobile phase (A/B, 80:20 (v/v), vortex mixed, filtered with a 0.45 μm syringe filter and transferred to autosampler vials. Twenty microlitres of aliquots were injected into the LC system for analysis.

2.4. Stability study

From the SPE procedure, methanolic solutions of the drugs were obtained. The stability of the drug in these solutions and effects of the interaction between drug, solvent and tube walls during the evaporation step was studied. Therefore, solutions of candesartan cilexetil were treated under different conditions, described as follows:

A. Methanolic solutions of candesartan cilexetil (20 μg/mL) were heated at 60 °C for 24 h in glass tubes (the same tubes used for the elution step).
B. Solutions of candesartan cilexetil in methanol (20 μg/mL) were evaporated under a stream of nitrogen at 60 °C in glass tubes.
C. Solutions of candesartan cilexetil in methanol (20 μg/mL) were evaporated under a stream of nitrogen at 60 °C in plastic tubes.
D. Solutions of candesartan cilexetil in acetonitrile (20 μg/mL) were evaporated under a stream of nitrogen at 60 °C in glass tubes.
E. Solutions of candesartan cilexetil in acetonitrile (20 μg/mL) were evaporated under a stream of nitrogen at 60 °C in plastic tubes.
F. Solutions of candesartan cilexetil in methanol (20 μg/mL) with ethylene glycol (10% in methanol) were evaporated (ethylene glycol remained in the tube) under a stream of nitrogen at 60 °C in glass tubes. Ethylene glycol was chosen due to its property of preventing adsorption processes on glass walls [10].

Residues were redisolved in solvent A/B (80:20) (v/v) and 20 μL were injected into the LC–DAD–MS system.

3. Results and discussion

The results of the stability study showed the formation of candesartan and a methyl derivate of this drug from candesartan cilexetil when methanolic solutions of this prodrug are evaporated to dryness under nitrogen in AR-Glas tubes. However, this does not occur when the methanolic solutions are heated but not evaporated to dryness, when methanol is replaced by acetonitrile or when the methanolic solutions are evaporated in plastic tubes.

Adding ethylene glycol avoids the evaporation to dryness of the methanolic solutions due to the high boiling point of this substance, but allows the hydrolysis to candesartan like in the other cases, and a transesterification is observed to hydroxylethylcandesartan. In this case, the methyl derivate was not found.

The degradation of candesartan cilexetil was studied by using HPLC with diode array detector. In Fig. 2, the UV chromatograms ($\lambda = 254$ nm) for the different experimental settings (mentioned in Section 2.4) are shown. The chromatographic peak obtained for candesartan cilexetil in methanolic solutions heated for 24 h in glass tubes (A) is decreased under B und F conditions (see Section 2.4). In these cases, two new peaks are found: one corresponding to the hydrolytic product candesartan (retention time: 11.9 min) and other one corresponding to the methyl derivate in case B (retention time: 13.8 min) and to the hydroxylethyl derivate in case F (retention time: 12.2 min). Approximately, between 70 and 80% of candesartan cilexetil was transformed to the hydrolysis and transesterification products.

The use of single quadrupole mass spectra was necessary to elucidate the structure of the new products by the molecular ion and the fragmentation pattern. In Fig. 3, the corresponding spectra for each compound are shown.

In order to study the effects of interactions of glass walls, different types of glass tubes and vials (including brown glass) were tested following the conditions described in Section 2. In these cases, no degradation of candesartan cilexetil was observed.

This fact suggested the presence of an unknown residue in the glass tubes which were usually used for the elution step during the SPE procedure. New glass tubes were then washed with acidic and basic solutions respectively, prior to rinsing them with deionised water. A glass control tube was also washed only with deionised water. With the basic conditioned glass

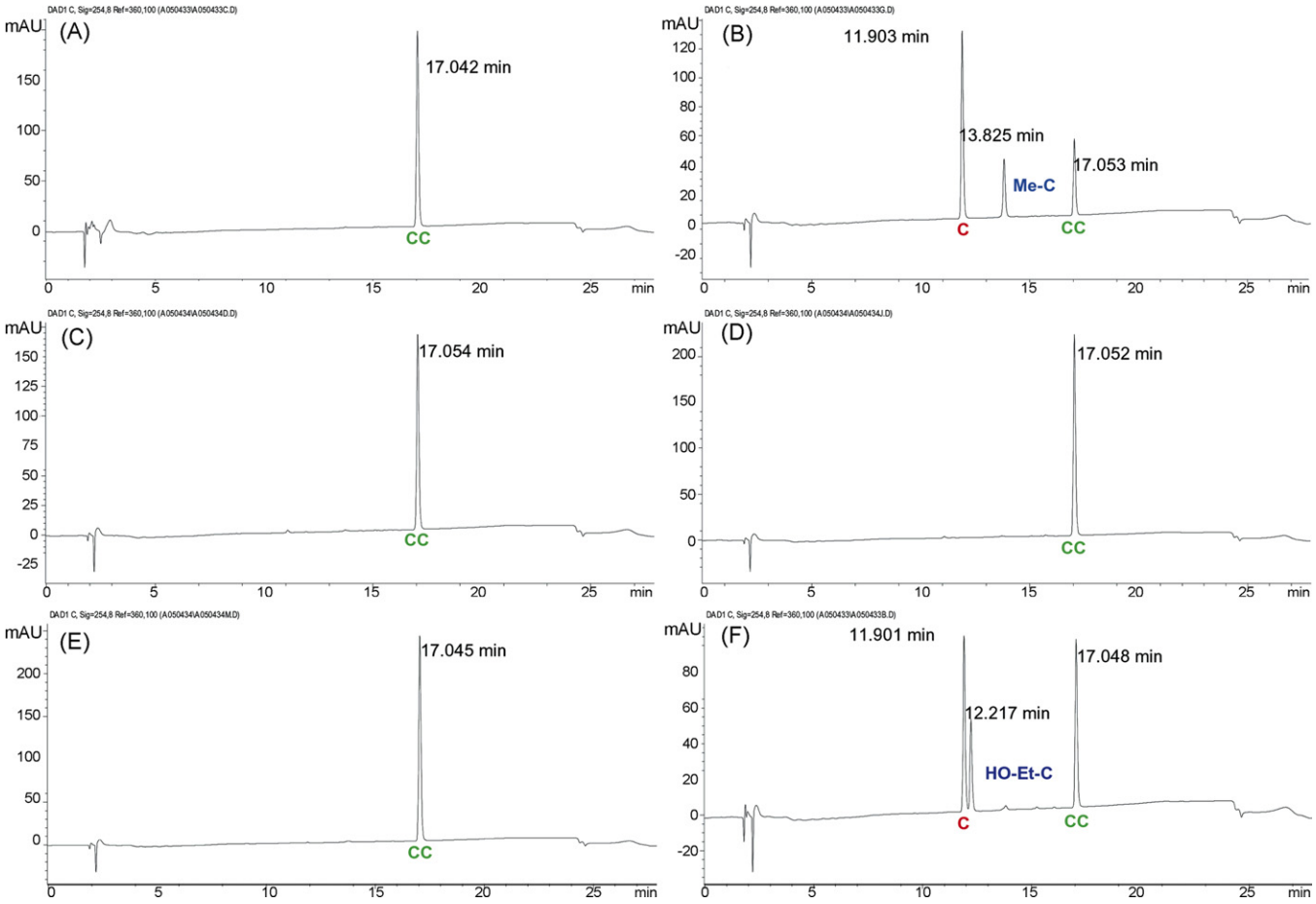

Fig. 2. The UV chromatograms (λ = 254 nm) for the described experimental procedure (A–F) and the retention times for each compound are shown. Only in the case of interaction between glass walls, methanol and evaporation, candesartan cilexetil (CC) is converted into candesartan (C) and other compounds like methylcandesartan (Me-C) or hydroxylethylcandesartan (HO-Et-C). The structures of these compounds were deduced from the mass spectra obtained in Q1 scan mode.

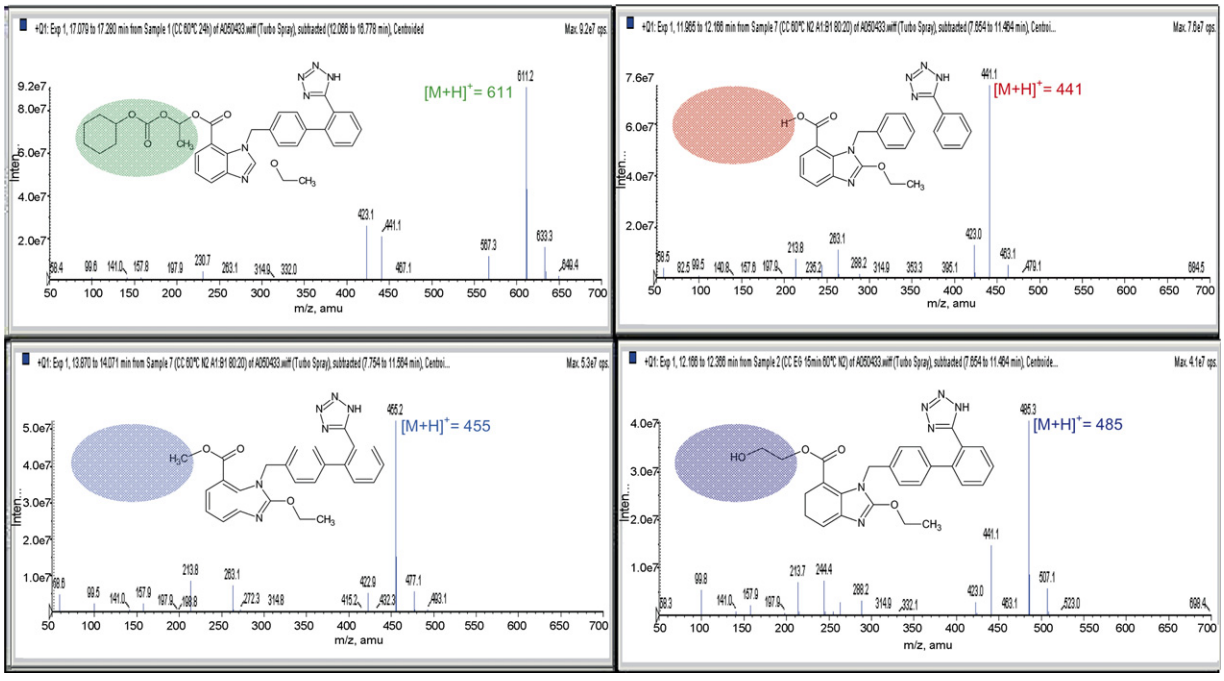

Fig. 3. The obtained mass spectra for candesartan cilexetil ([M + H]$^+$ = 611), candesartan ([M + H]$^+$ = 441), methylcandesartan ([M + H]$^+$ = 455) and hydroxylethylcandesartan ([M + H]$^+$ = 485), using a declustering potential of 20 V, are shown.

tubes, hydrolysis and transesterification were observed. After washing with water or acidic solution, the prodrug became stable.

Hydrolysis and transesterification reactions occurred in methanolic medium and glass surfaces when the solution was evaporated, which suspected the presence of some kind of basic residue in the glass wall, whose concentration, per se, was not enough to unleash these reaction; but during the evaporation step, the adequate concentration was achieved and the hydrolysis and transesterification processes were carried out.

4. Conclusions

The prodrug candesartan cilexetil undergoes base catalysed hydrolysis and transesterification mediated by glass walls when it is evaporated from methanolic solutions under nitrogen stream. These phenomena do not occur when glass walls are rinsed with acidic solutions prior to the evaporation step, but they are increased by the use of basic rinse solutions. We recommend rinsing with acidic solutions prior to use and/or use other kind of glass such as borosilicate.

The formation of candesartan and its ester can be explained as a basic hydrolysis of candesartan cilexetil and a transesterification reaction of the prodrug catalysed by the surface of glass tubes, due to any kind of basic contamination in the tubes.

Differences in this process were noticeable when glass from different lots or even tubes from the same lot were used, so we recommend testing for these phenomena when labile drugs are analysed. Examples for other labile esters are cocaine, benzoylecgonine, THC-COOH-glucuronide and ACE inhibitors.

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