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An overview of chromatographic methods coupled with mass spectrometric detection for determination of angiotensin-converting enzyme inhibitors in biological material

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Abstract

Gas and liquid chromatography–mass spectrometry (GC–MS, LC–MS) methods for the determination of angiotensin-converting enzyme inhibitors (ACEIs) and their metabolites in biological material have been reviewed. Since 1980s those hyphenated techniques have been applied to quantitate ACE inhibitors and the dynamic increase in the number of relevant publications can be observed in recent years. Although most of the methods available in the literature were analyses of plasma or serum, assays of blood and urine were also included. Additionally, sample pretreatment methods, separation conditions and ionization modes were overviewed. Some information on chemical structures, *cis–trans* izomerization and stability of compounds in question was also included. Most of the reported methods were successfully applied to the pharmacokinetic studies in humans.

Keywords: Angiotensin-converting enzyme (ACE) inhibitors and their metabolites; Gas chromatography–mass spectrometry (GC–MS); Liquid chromatography–mass spectrometry (LC–MS); Biological material; Bioanalysis

1. Introduction

The angiotensin-converting enzyme inhibitors are widely used in the management of essential hypertension, stable chronic heart failure, myocardial infarction and diabetic nephropathy [1–3].

Angiotensin-converting enzyme plays a central role in a cascade of proteolytic reactions, which ultimately control the levels of angiotensin II, a potent vasoconstrictor [4]. At first, renin cleaves the inactive substrate angiotensinogen to the decapeptide angiotensin I. In turn, angiotensin-converting enzyme catalyses the transformation of angiotensin I into angiotensin II, the active octapeptide of the renin-angiotensin system (Fig. 1). ACE also inactivates bradykinin, a potent vasodilating agent. ACE inhibitors evoke the opposite action.

In 1977 captopril – the first from the group of ACE inhibitors – was synthesized followed by a number of new compounds of similar activity and a longer half-life [3,5]. ACEIs of interest were selected according to WHO ATC index 2006 (group C09AA–ACE inhibitors, plain) [6] and were listed below: benazepril (BEN), captopril (CAP), cilazapril (CIL), delapril (DEL), enalapril (ENA), fosinopril (FOS), imidapril (IMI), lisinopril (LIS), moexipril (MOE), perindopril (PER), quinapril (QUI), ramipril (RAM), spirapril (SPI), temocapril (TEM), trandolapril (TRA) and zofenopril (ZOF). Respective active

Abbreviations: ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; ACN, acetonitrile; ATC, anatomical therapeutic chemical; C18, octadecyl; C8, octyl; CI, chemical ionization; C_{max}, peak plasma concentration; CN, cyanopropyl; EA, ethyl acetate; EMEA, European Agency for the Evaluation of Medicinal Products; EI, electron impact; ESI, electrospray ionization; FDA, U.S. Food and Drug Administration; GC, gas chromatography; HFB, heptafluorobutyrate; HLB, hydrophilic–lipophilic balance copolymer; HPLC, high performance liquid chromatography; LC, liquid chromatography; LLE, liquid–liquid extraction; LLOQ, lower limit of quantification; MBTFA, *N*-methylbis(trifluoroacetamide); MeOH, methanol; MS, mass spectrometry; MTBE, methyl *tert*-butyl ether; N/A, not available; NEM, *N*-ethylmaleimide; NICI, negative ion chemical ionization; PFB, pentafluorobenzyl; PGC, porous graphitized carbon; PP, protein precipitation; r.t., room temperature; SPE, solid-phase extraction; TFA, trifluoroacetic acid; TIS, turbo ion spray; TMSDM, trimethylsilyldiazomethane; ULOQ, upper limit of quantification; WHO, World Health Organisation

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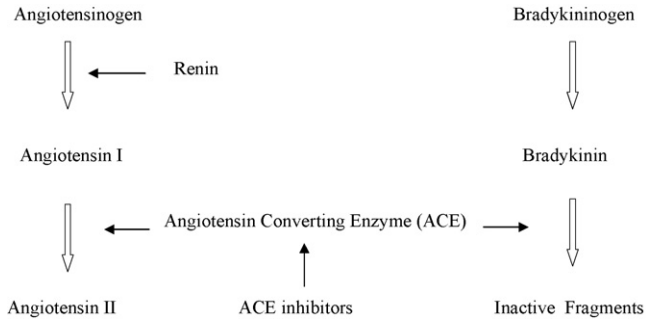

Fig. 1. The formation of angiotensin II, showing the role of angiotensin-converting enzyme.

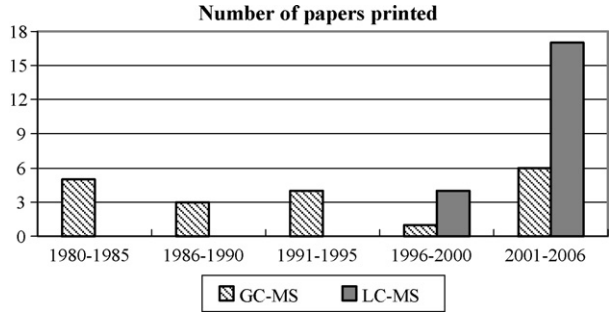

Fig. 2. Chronological comparison of papers on hyphenated chromatographic determinations of ACEIs in biomatrices.

metabolites were abbreviated by adding an -at suffix (e.g. BEN-at for benazeprilat). CAP and LIS are active as such, while most other ACE inhibitors are prodrugs [3], which require hepatic activation to form pharmacologically active metabolites.

Determination of ACE inhibitor levels in biological fluids was based either on radioimmunoassay, fluoroimmunoassay or, indirectly, on assays of enzymatic activity of ACE. Enzymatic activity of ACE was usually determined with suitable substrates by spectrophotometry or fluorometry, or with the use of a radiolabelled substrate. Since 1980s the hyphenated techniques have been applied to determine ACE inhibitors in biological material and the dynamic increase in the number of relevant publications can be observed in recent years (Fig. 2). The use of hyphenated techniques offers substantially improved possibilities of pharmacologic and pharmacokinetic studies.

The main purpose of the present review was to collect, focus on and summarize the chromatographic methods coupled with mass spectrometric detection for the determination of ACE inhibitors in biological fluids [7–47]. Some information on chemical structures, *cis–trans* izomerization and stability of compounds in question was also included.

2. Chemical properties

2.1. Chemical structures and chirality

In the literature, there exists a single classification of ACEIs chemical structures [48]. The first class includes sulfhydryl-containing drugs (e.g. CAP and its analogues), the second – carboxyalkyldipeptides (e.g. ENA and its analogues), and the third class – phosphorus-containing drugs (FOS).

In the present review another criterion has been proposed. More balanced distribution of marketed ACE inhibitors was based on the identification of three common main structures. Thus, the ACEIs were divided into three groups, i.e.: derivatives of proline (group I), of *N*-[(1-methyl-2-oxo)ethyl]glycine ethyl ester (group II) and of ethyl 2-amino-4-phenylbutyrate (group III). The chemical structures are shown in Tables 1–3, respectively.

Each molecule of the compounds in question contains at least two chirality centers, located both in the base structure and in the side chains. It has been reported that S-stereoisomers are biologically active, while R-stereoisomers exhibit no ACE inhibiting activity [48]. Thus the stereochemical purity of ACEIs during drug synthesis should be monitored, e.g. by HPLC method [49].

2.2. The cis–trans isomerization

Influence of *cis–trans* isomerization on the chromatographic behaviour of ACE inhibitors (LIS and ENA) was described by Tsakalof et al. [30] and Kocijan et al. [50]. The configuration of a proline peptide bond in LIS and ENA molecules can be either *cis* or *trans*. Generally *trans*-configuration is preferential in peptides, but in proline-containing ones *cis*-configuration is likely to occur. The *cis–trans* interconversion may appear due to the reduced barrier height, but the rotation around the peptide bond is restricted because of the partial double bond character. During chromatographic separation of ENA and the related compounds, the isomer interconversion occurs influencing the peak shape or even splitting the peak. This, in turn, can result in the misinterpretation of the peak identity. It was reported that the peak shape strongly depends on temperature, pH and flow rate: ACEIs elute as a single sharp peak at a high column temperature (80 °C) and low pH (pH 2). The problems of ACEIs *cis–trans* isomerization were also discussed in other papers [51,52].

3. Methods

A wide range of bioanalytical techniques was used in previously described assays. Enzymatic [53,54] or fluoroenzymatic [55] reactions and radioimmunoassays [56–61] were the methods of choice in the earlier papers (1980s, and early 1990s). Although very sensitive, these methods were rather expensive and the achievement of desirable precision sometimes required a triple sample analysis. Moreover, it was difficult to determine prodrug and metabolite concentrations in a single analysis. On the other hand, many developed HPLC assays with UV–vis [62–68], fluorescence [69–73], electrochemical [74,75] and voltammetric [76,77] detection were more specific. Most of them required a complicated sample pretreatment and time-consuming chromatographic separation, but the main disadvantage was the sensitivity, which was usually not sufficient for the pharmacokinetic studies. Single papers on the application of thin-layer radiochromatography [78] and capillary electrophoresis with laser-induced fluorescence detection [79] were also retrieved.