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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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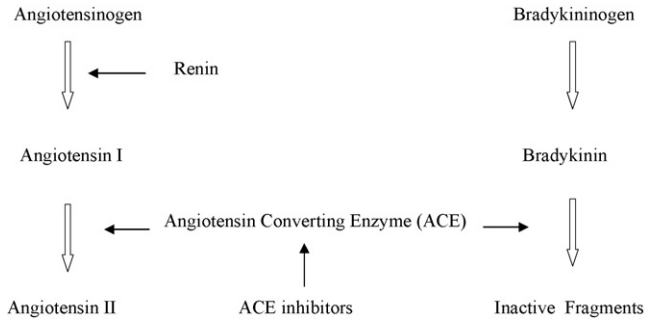


Fig. 1. The formation of angiotensin II, showing the role of angiotensin-converting enzyme.

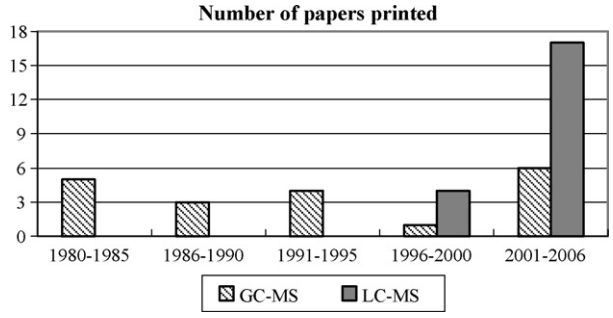


Fig. 2. Chronological comparison of papers on hyphenated chromatographic determinations of ACEIs in biomatrices.

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].

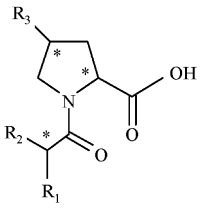
2.2. The cis–trans isomerization

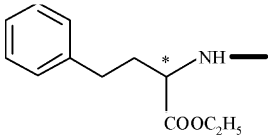
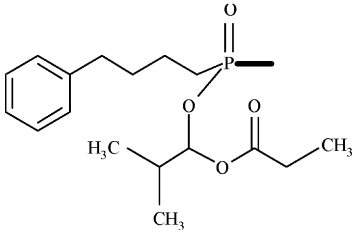
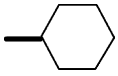
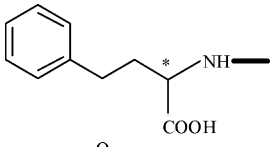
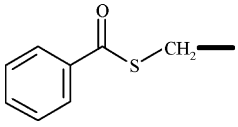
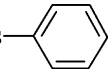
Influence of *cis–trans* isomerization on the chromatographic behaviour of ACE inhibitors (LIS and ENA) was described by Tsakalof et al. [30] and Kocijan et al. [50]. The configuration of a proline peptide bond in LIS and ENA molecules can be either *cis* or *trans*. Generally *trans*-configuration is 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.

Table 1
ACEIs chemical structures – group I



Code	R_1[a]	R_2	R_3[a]
CAP	—CH_3	$HS–CH_2$—	—H
ENA	—CH_3		—H
FOS	—H		
LIS	—$C_4H_8–NH_2$		—H
ZOF	—CH_3		—S 

A bold bond in the structures above marks a place of binding with the general structure. *Chirality center.
[a] Lack of chirality when R_1 and/or R_3 is H-atom.

From the very beginning, the hyphenated techniques were widely applied to ACEIs determination in biological fluids. The use of GC–MS and LC–MS not only replaced the hazardous and expensive radiolabelled compounds in many bioanalytical laboratories, but also enabled the concurrent determination of prodrugs and their metabolites. The gas chromatography–mass spectrometry methods were developed upon the launch of the reviewed drugs in 1980s. GC–MS provided reliable results after a short-time single analysis, but still most of the sample pretreatment methods included both extraction and sophisticated derivatization steps.

Mass spectrometry coupled with liquid chromatography was first applied to ACEIs determination in 1999 [27]. The main reasons for LC–MS application in bioanalysis was the improved selectivity and sensitivity, which resulted in the simplified sample pretreatment and shorter analysis time.

Another possible method for the detection and identification of natural ACE inhibitors found in food is on-line liquid chromatography–biochemical detection (LC–BCD) coupled to electrospray mass spectrometry [80].

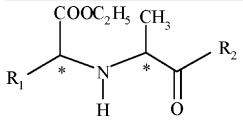
Published hyphenated chromatographic methods (in English) were retrieved using Entrez PubMed search system [81]. Only the completely described GC–MS and LC–MS assays were overviewed. It was found that the reported methods are comparable in each group of compounds, irrespective to the classification applied. Thus, the reviewed methods were summarised in Table 4 and listed alphabetically by a drug name. Horizontal lines inside the row indicate different data recorded for the drug and the respective metabolite (e.g. values of LLOQ). Among 16 ACE inhibiting substances reviewed no hyphenated chromatographic methods were found in the case of CIL and TRA measurements in biological fluids.

3.1. Sample pre-treatment

Although mass detection is very selective, a need for sample preparation cannot be omitted. Not only can the interfering endogenous substances from complex biomatrices be separated, but also the analyte concentration may be enhanced. Sample preparation techniques applied in GC and LC mass spectrometric methods were presented graphically (Figs. 3 and 4, respectively).

It can be observed that the GC–MS methods involved more extensive sample pretreatment. The analytes usually

Table 2
ACEIs chemical structures – group II



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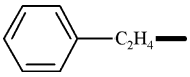
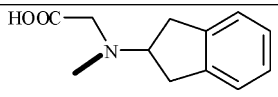
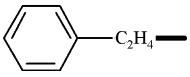
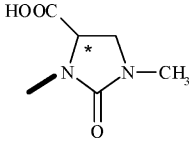
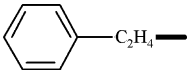
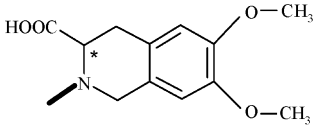
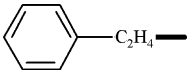
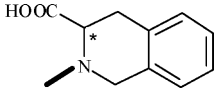
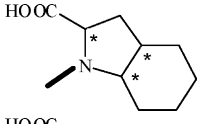
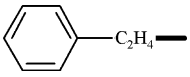
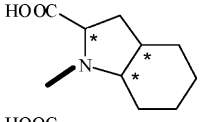
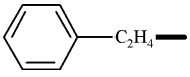
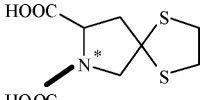
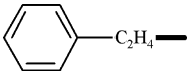
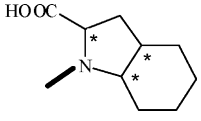
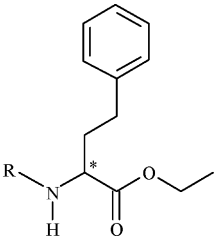
DEL		
IMI		
MOE		
QUI		
PER	C_3H_7	
RAM		
SPI		
TRA		

A bold bond in the structures above marks a place of binding with the general structure. *Chirality center.

Table 3
ACEIs chemical structures – group III



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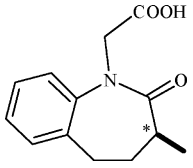
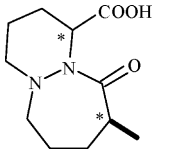
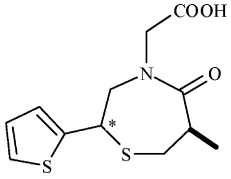
BEN	
CIL	
TEM	

A bold bond in the structures above marks a place of binding with the general structure. *Chirality center.

required a derivatization step into volatile and thermally stable molecules. Sample preparation procedures described in nearly a half of reviewed methods were complex, including liquid–liquid extraction, protein precipitation, solid-phase extraction and column chromatography steps combined in various order. Among more straightforward methods, SPE followed by derivatization was more popular than LLE combined with derivatization.

The most popular sample preparation technique among liquid chromatography–mass spectrometry methods was solid-phase extraction, sometimes combined with protein precipitation or derivatization. The octadecyl and HLB cartridges were of the first choice, but a single assay was based on cyclohexyl cartridges. Compounds of interest were usually eluted with MeOH.

The simple protein precipitation and liquid–liquid extraction were reported in case of some LC–MS methods. Ethyl acetate was the most popular among various organic solvents used as extraction media (e.g. MTBE, diethyl ether, dichloromethane, dichloroethane, isopropanol, hexane, toluene). In contrast to GC–MS, a single paper reporting derivatization in a LC–MS sample pre-treatment was retrieved [23].

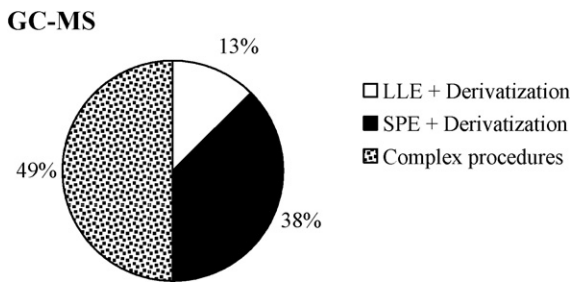


Fig. 3. Sample pretreatment techniques applied in GC–MS papers.

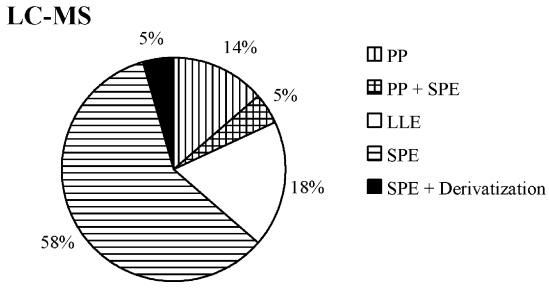


Fig. 4. Sample pretreatment techniques applied in LC–MS papers.

Table 4
Hyphenated chromatographic determination of ACEIs in biological material

ACEI	Matrix	Analytical technique	Sample pretreatment	Chromatographic column	Chromatographic conditions	LLOQ (ng/mL)	ULOQ (ng/mL)	Monitored ions (*m/z*)	Reference
BEN	Human plasma, urine	GC–MS EI (+)	*Plasma*: XAD-2 column, elution: MeOH; *urine*: Extrelut-1 column, elution: CH_2Cl_2–EtOH (95:5, v/v); both: derivatization with diazomethane, acid–base partition	12.5 m × 0.2 mm	Carrier gas: helium; oven temp.: 0.5 min–210 °C, 50 °C/min to 290 °C; run time: 5 min	2.3[a]; 4.6[b]	92[a]; 230[b]	365	[7]
BEN-at						2.6[a]; 21.0[b]	103[a]; 523[b]	365	
BEN and BEN-at	Human plasma	GC–MS EI (+)	SPE C18 96-well, elution: MeOH, derivatization with TMSDM, acid–base partition, LLE: MTBE	15 m × 0.25 mm, 25 μm	Carrier gas: helium; oven temp.: 190–290 °C, 30 °C/min; run time: 6 min	2.5	1000	365, 365	[8]
BEN and BEN-at	Human plasma	LC–MS ESI (+)	PP with MeOH and ACN	C18, 250 mm × 4.6 mm, 5 μm (temp., 30 °C)	Gradient—A: ACN/B: 0.1% HCOOH; flow rate: 0.8 mL/min; run time: 15 min	6.7	667	425.5, 397.5	[9]
BEN and BEN-at	Human plasma	LC–MS ESI (+)	SPE HLB, elution: MeOH	PGC, 125 mm × 2.1 mm, 5 μm	ACN–0.3% HCOOH (55:45, v/v); flow rate: 0.15 mL/min; run time: 12 min[c]	5.0	500	425.0, 397.0	[10]
CAP	Human blood	GC–MS (+)	Derivatization with NEM, XAD-2 column, elution: EA	0.7 m × 2 mm	Carrier gas: helium; column temp.: 200–280 °C	16.5	2000	230	[11]
CAP	Human blood, urine	GC–MS CI (+)	*Blood*: XAD-2 resin after derivatization with NEM, elution: EA [11]; *Urine*: LLE: EA	0.8 m × 2mm	Carrier gas: helium; column temp.: 260 °C; injector temp.: 290 °C	N/A	1500	230.1	[12]
CAP	Dog blood, rat urine	GC–MS (+)	*Blood*: PHP-LH-20 column 20 mm × 5 mm after derivatization with NEM, elution: 7% acetic acid in ethanol; *Urine*: elution: *n*-hexane	1 m × 3 mm	Carrier gas: helium; column temp.: 258 °C; injector and ion source temp.: 270 °C	N/A	N/A	732	[13]
CAP	Human plasma	GC–MS	XAD-2 column after derivatization with NEM, elution: EA	0.8 m × 2 mm	Carrier gas: helium; column temp.: 260 °C; injector temp.: 290 °C	20.0	N/A	230.1	[14]
CAP[d]	Human plasma, blood	GC–MS EI (+)	XAD-2 column, elution: EA	0.8 m × 2 mm	Carrier gas: methane; injector temp.: 290 °C; run time: 2.5 min	N/A	N/A	N/A	[15]
CAP	Human blood	GC–MS NICI (−)	LLE: EA, derivatization with PFB and diazomethane	15 m × 0.25 mm, 0.25 μm	Carrier gas: helium; injector temp.: 290 °C	0.5	300	341	[16]
CAP	Human plasma	LC–MS/MS ESI (+)	LLE: diethyl ether–CH_2Cl_2 (70:30, v/v)	C8, 150 mm × 4.6 mm, 5 μm (r.t.)	ACN–water (70:30, v/v) + TFA (0.013 M); flow rate: 1.5 mL/min (split); autosampler temp.: 4 °C	25.0	3000	218.0 > 171.6	[17]
CAP	Human plasma	GC–MS EI (+)	LLE: CH_2Cl_2, reextraction $NaHCO_3$, acidification, back-extraction to CH_2Cl_2, derivatization with PFB	25 m × 0.2 mm, 0.33 μm	Carrier gas: helium; injector temp.: 160–280 °C at 50 °C/min; oven temp.: 80–260 °C at 40 °C/min, 260–290 °C at 10 °C/min	10.0	5000	294, 396	[18]
DEL, DEL-at, M2 and M3	Human plasma	LC–MS/MS ESI (+)	SPE C18, elution: MeOH	C18, 70 mm × 2 mm, 5 μm	ACN–water–TFA (50:50:0.06, v/v/v)	10.0	1000	N/A	[19]
ENA and ENA-at	Human plasma	LC–MS/MS TIS (+)	SPE HLB, elution: MeOH	C18, 50 mm × 2.1 mm, 3.1 μm	0.4% Acetic acid in water–ACN (38:62, v/v); flow rate: 0.28 mL/min	1.0	200	377.1 > 234.2; 349.0 > 206.1	[20]
ENA-at	Human plasma	GC–MS EI (+)	SPE C18, derivatization with methyl iodide	30 m × 0.20 mm, 0.33 μm	Carrier gas: helium; oven temp.: 1 min–200, 30 °C/min to 300	5.0	160	220	[21]
ENA and ENA-at	Human plasma	LC–MS/MS TIS (+)	LLE: EA	C18, 150 mm × 4.6 mm, 5 μm (temp., 30 °C)	MeOH–water–HCOOH (70:30:1, v/v/v); flow rate: 0.6 mL/min; run time: 3.5 min	0.1	100	377 > 234; 349 > 206	[22]
ENA and ENA-at	Human plasma	LC–MS ESI (+)	SPE C18, elution: ACN–ammonium acetate (0.05 M)–HCOOH (90:10:0.5, v/v/v), derivatization with diazomethane	C18, 150 mm × 4.6 mm, 5 μm (temp., 80 °C)	Gradient—A: MeOH–ammonium acetate (0.1 M)–HCOOH (95:5:0.5, v/v/v); B: water–ammonium acetate (0.1 M)–HCOOH (95:5:0.5, v/v/v), *t* = 0 55% A, *t* = 8 75% A; flow rate: 0.7 mL/min; run time: 14 min[c]	0.5	200	391; 377	[23]
FOS-at	Human serum	LC–MS/MS ESI (+)	SPE C18 96-well, elution ammonium acetate (pH 5.5; 0.01 M)	C8, 50 mm × 2 mm, 3 μm	20%A–80%B; A: MeOH–ammonium acetate in water (pH 5.5; 0.01 M) (25:75, v/v); B: ammonium acetate (pH 5.5; 0.01 M) in MeOH; flow rate: 0.3 mL/min; run time: 2 min	2.0	500	436 > 390	[24]
FOS and FOS-at	Human serum	LC–MS/MS TIS (+)	SPE cyclohexyl, elution: ammonium acetate (0.01 M) in MeOH	C18, 50 mm × 2 mm, 5 μm	Gradient—A: water–MeOH containing ammonium acetate (pH 5.5; 0.01 M) (75:25, v/v); B: ammonium acetate (0.01 M) in MeOH; flow rate: 0.2 mL/min; run time: 10 min	1.17	300	581.3 > 436.2; 453.2 > 390.2	[25]
M2, M3, M4 of IMI	Human plasma, urine	GC–MS/MS EI (+)	SPE C18, elution: M2—HCl (0.1 M), M3 and M4—80% ethanol–HCl (5 mM), derivatization with PFB bromide or HFB anhydride	15 m × 0.53 mm, 1.5 μm	Carrier gas: helium; oven temp.: 1 min–200 °C, 5 °C/min to 245 °C[a] and to 230 °C[b]	5.0[a]; 10.0[b]	100[a]; 2000[b]	520 > 295; 459 > 386; 611 > 386	[26]
IMI and IMI-at	Human plasma	LC–MS/MS ESI (+)	PP with 6% $HClO_4$; SPE HLB, elution: MeOH	C18, 100 mm × 2.1 mm, 3.5 μm (temp., 40 °C)	ACN–0.05 % HCOOH (1:3, v/v); flow rate: 0.2 mL/min; run time: 6 min; autosampler temp.: 10 °C	0.2	50	406 > 234; 378 > 206	[27]

IMI	Human plasma	LC–MS ESI (+)	SPE C18, elution: MeOH	C18, 150 mm × 2.1 mm, 3.5 μm (temp., 40 °C)	ACN–0.1% HCOOH (67:33, v/v); flow rate: 0.3 mL/min	0.5	100	406	[28]
LIS	Human plasma	GC–MS NICI (−)	SPE C18, elution: MeOH, derivatization with MBTFA and PFB, Si column	15 m × 0.25 mm, 0.25 μm	Carrier gas: helium; injector temp.: 280 °C; oven temp.: 180–315 °C at 40 °C/min	0.4[e]	100[e]	776	[29]
LIS	Human serum	LC–MS ESI (+)	SPE C18, elution: MeOH	C18, 250 mm × 3.2 mm, 5 μm	Ammonium formate (pH 4.3; 0.05 M)–ACN–MeOH (72:7:21, v/v/v); flow rate: 0.6 mL/min (split); run time: 6 min	6.0	150	406.5	[30]
LIS	Human plasma	LC–MS ESI (+)	SPE, elution: MeOH	C18, 150 mm × 3.9 mm, 5 μm	1% Acetic acid–MeOH (50:50, v/v); flow rate: 0.5 mL/min; run time: 5 min	10.0	500	405.7	[31]
LIS	Human plasma	LC–MS/MS ESI (+)	SPE HLB, elution: MeOH	C8, 150 mm × 4.6 mm (temp., 40 °C)	ACN–water (60:40, v/v) + acetic acid (0.02 M) + TFA (4.3 mM); flow rate: 0.5 mL/min (split); run time: 6.5 min	2.0	200	406.3 > 84.3	[32]
LIS	Human plasma	LC–MS/MS	PP with TFA	C8(2), 150 mm × 2 mm	MeOH–0.1% HCOOH (50:50, v/v); flow rate: 0.25 mL/min	1.28	160	N/A	[33]
LIS	Human plasma	LC–MS/MS	SPE, elution: MeOH	CN, 250 mm × 4.6 mm, 5 μm (temp., 50 °C)	0.4% TFA–ACN (43:57, v/v); flow rate: 1.0 mL/min (split)	N/A	N/A	406.3 > 84.1	[34]
LIS	Human plasma	LC–MS/MS ESI (+)	LLE: isopropanol–EA (1:2, v/v)	CN, 150 mm × 2.1 mm, 5 μm	MeOH–0.1% HCOOH in water (50:50, v/v); flow rate: 0.2 mL/min	0.78	100	406.1 > 246.0	[35]
MOE and MOE-at	Human plasma	GC–MS NICI (−)	SPE C18, elution: CH_2Cl_2–MeOH (2:1); methylation; acid–base partition; TFA	10 m × 0.32 mm, 0.25 μm	Carrier gas: helium; injector temp.: 70–300 °C at 10 °C/s; oven temp.: 150–300 °C at 25 °C/min; run time: 7.5 min	0.5	300	302; 288	[36]
PER	Human plasma	LC–MS/MS TIS (−)	SPE HLB, elution: MeOH	C8, 30 mm × 2.1 mm, 3.5 μm (temp., 35 °C)	0.1% aqueous ammonia–MeOH (20:80, v/v); flow rate: 0.3 mL/min; run time: 1.5 min	0.5	350	368.1 > 168.1	[37]
PER-at						0.3	40	339.3 > 168.1	
QUI and QUI-at	Human plasma, urine	GC–MS NICI (–)	SPE C18, elution: $CHCl_3$–2-butanol (2:1, v/v), derivatization with PFB-TFA	10 m × 0.32 mm, 0.17 μm	Carrier gas: helium Oven temp.: 2 min–200 °C, 70 °C/min to 300 °C	0.05[a]; 0.5[b]	30[a]; 1000[b,f]	533; 685	[38]
QUI metab. I + II[g]	Human plasma, urine	GC–MS EI (+)	LLE: hexane–dichloroethane (1:1, v/v) under acidic conditions, derivatization with PFB, Si column	10 m × 0.32 mm, 0.52 μm	Carrier gas: helium; oven temp.: 1 min–272 or 286 °C (I or II), 16 °C/min to 310 or 316 °C (I or II, respectively); run time: 4 min	0.2[a]	10[a,b,f]; 1.0[b]	316; 468	[39]
QUI and QUA-at	Human plasma	LC–MS ESI (+)	SPE C18, elution: CH_2Cl_2–MeOH (2:1, v/v)	CN, 33 mm × 4.6 mm, 3 μm	MeOH–0.0018% HCOOH (8:2, v/v); flow rate: 0.35 mL/min, run time: 11 min	5.0; 10.0	500; 1000	439.15; 411.20	[40]
RAM and RAM-at	Human plasma	LC–MS/MS TIS (+)	SPE HLB, elution: MeOH	C8, 50 mm × 2.1 mm, 5 μm	ACN-MeOH–0.1% HCOOH (4:4:5, v/v); flow rate: 0.21 mL/min, run time: 2.5 min	0.5	250	417.2 > 234.1; 389.2 > 206.1	[41]
RAM	Human plasma, urine	GC–MS EI (+)	SPE C18, derivatization with TMSDM and TFA	10 m × 0.25 mm, 0.15 μm	Carrier gas: helium; injector temp.: 260 °C	0.4	125	330	[42]
RAM-at							117	316	
RAM	Human serum	LC–MS/MS ESI (+)	PP with MeOH	C18, 100 mm × 2.1 mm, 3 μm	0.1% HCOOH:MeOH (25:74, v/v); flow rate: 0.2 mL/min	0.1	100	417.3 > 234.3	[43]
RAM-at						0.25	500	389.3 > 206.2	
SPI and SPI-at	Human plasma	GC–MS	SPE C18, elution: EA–MeOH (3:2, v/v), derivatization with TMSDM	15 m × 0.25 mm	Carrier gas: helium; oven temp.: 0.5 min–200 °C (isothermal), 30 °C/min to 300 °C, 5.5 min 300 °C; run time: 9.3 min	2.5	500	234.15; 220.15	[44]
TEM and TEM-at	Human plasma, urine	GC–MS NICI (−)	SPE C18, elution: MeOH, derivatization with diazomethane, Si column, elution: $CHCl_3$, derivatization with TFA	10 m × 0.25 mm, 0.25 μm	Carrier gas: helium; oven temp.: 1 min–200 °C, 30 °C/min to 280 °C; run time: 6 min	1.56	100[f]	288; 293	[45]
ZOF	Human plasma	LC–MS/MS TIS (−)	LLE: toluene	C18, 75 mm × 4.6 mm, 3 μm	Gradient—A: ACN; B: ammonium acetate (pH 4.5; 0.026 M); flow rate: 0.4 mL/min, run time: 9 min	1.0	300	428 > 137	[46]
ZOF-at						2.0	600	449 > 290	

[a] Plasma.
[b] Urine.
[c] Data from a chromatogram.
[d] Unchanged CAP, disulfide dimer and mixed disulfides with endogenous thiol-containing compounds.
[e] Presented in a previous paper [47].
[f] Not clearly presented.
[g] Metabolites other than QUA-at (dioxopiperazine); the abbreviations were explained in paragraphs 1 (drug names) and in the abbreviations section (others).

3.2. Separation conditions

Since 1980s – thanks to advances in instrumentation – an improvement in gas chromatographic separation can be observed. According to the earlier papers concerning GC–MS, the packed columns of the length not exceeding 1 m were employed (in six of seven papers from 1980s). In 8 of 11 more recent publications, more efficient capillary columns of 10–15 m in length were employed, whereas those of 25–30 m were rarely used (three cases). Apart from one assay with the use of methane, helium was employed as a carrier gas.

Among 19 liquid chromatography assays reviewed, generally the reverse phase mode was the method of choice. The most popular column packages were C18 and C8, although CN and PGC were also used. To shorten the analysis times, a column below 100 mm in length was used in more than one third of cases (7 of 19), whereas a 250 mm column was used three times. The column diameter depended strongly on the column length and amounted to 2.0–2.1 mm in assays with short LC–MS dedicated columns. Diameters of a longer HPLC column were from 3.9 to 4.6 mm. Generally, the improved chromatographic separation prevents the occurrence of the matrix effect, as further discussed in the next paragraph.

3.3. Ionization and fragmentation

The molecules of ACE inhibitors contain both nitrogen atoms and carboxylic groups, thus both positive and negative ionization is possible, the positive ionization being observed more frequently (Fig. 5). Ion sources included electron impact and negative ion chemical ionization in GC–MS and both electrospray ionization and turbo ion spray in LC–MS assays. Due to the polar structure of the drugs in question, no atmospheric pressure chemical ionization methods were employed to determine ACEIs in the biological material.

The present authors' experience in the field of ACEIs analysis indicates that matrix components strongly influence LC–MS detection of these compounds. This phenomenon, called the matrix effect, was widely described in the literature [82–87]. In the case of liquid chromatography–mass spectrometry procedures, the matrix effect should be investigated, especially when the matrix source used during the method validation and application is changed [88]. There were a few of the papers reporting LC–MS methods presented in this review that provided any information in this field.

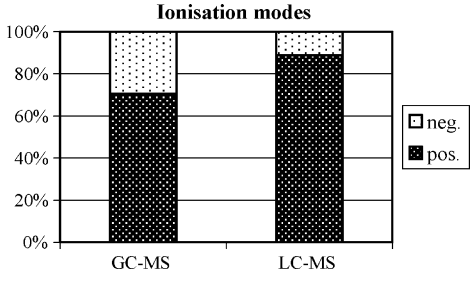


Fig. 5. Ionization modes.

The mass spectral fragmentation reactions of ACEIs were investigated by Burinsky and Sides [89]. They explained specific rearrangement of LIS and noted that ACEIs ester molecules produced unexpectedly complex dissolution spectra. On the other hand, compounds having carboxylic acid groups at both C-terminus and N-terminus blocking groups displayed spectra with a single signal only, which is perfect for analytical applications.

3.4. Internal standard

The internal standard (I.S.) calibration was found to be the method of choice. Two of 40 methods presented in Table 4 did not employ I.S. [20,40]. Usually, another ACEI, the respective metabolite, derivate or labelled analogue were used as I.S.

3.5. Stability

As most of the reviewed methods were applied to pharmacokinetic studies, the stability issues were of great importance. Both FDA and EMEA requirements for validation of bioanalytical methods include: short-term (in biomatrix at r.t.), long-term (in biomatrix at storage conditions), post-preparative, after three freeze–thaw cycles, and solutions stability studies [88,90]. A few papers presented all the respective data, while other included the selected results or omitted the stability issues. Published (incomplete) data suggest that ACEIs have to be considered as stable in the following conditions: 4–24 h at r.t. in the biological material, up to 7 months at −20 °C as well as after 3 freeze–thaw cycles at −20 °C. The extracted samples were generally stable at r.t. for at least 24 h in an autosampler, but post-preparative stability of CAP, LIS, PER and RAM was confirmed at lower temperatures (4–10 °C). The stock solutions were stable during 4–24 h at r.t., over 1 month at 4 °C and for 1 year at −40 °C.

Photosensitivity of QUI and QUI-at has been reported [65], while one of the CAP extraction methods was performed under the light of a sodium lamp [17]. To prevent CAP from disulfide derivative formation, *N*-ethylmaleimide was added to the stock solution of this ACEI and to plasma collection tubes [18].

4. Determination of individual ACEIs and their metabolites

4.1. Benazepril (BEN)

The reviewed BEN assays included the simultaneous determination of its active metabolite BEN-at. The described determinations were performed in human plasma samples [8–10] or both in human plasma and urine [7]. The GC–MS methods were slightly more sensitive, with LLOQs ca. 4 ng/mL lower than in LC–MS methods, but this difference does not seem to be significant. The sample pretreatment in LC–MS assays, including protein precipitation [9] or simple SPE [10], was definitely easier than in GC–MS methods. To prepare samples for GC–MS analysis, plasma was extracted on C18 cartridges [8] (or XAD-2 column [7]) then the derivatization with TMSDM [8] (or diazomethane [7]) and acid–base partition was performed. Finally, the compounds of interest were extracted with TMBE

[8] (or diethyl ether-dichloromethane [7]), evaporated and dissolved in toluene to inject onto the chromatograph. In both methods positive ionization was used. Contrary to sample preparation, the GC–MS methods run-time was shorter than in the LC–MS case. The stock solutions of BEN in MeOH and BEN-at in 1.2% ammonia–MeOH (6:4, v/v) were found to be stable for 1 year at −40 °C [9]. To prevent enzymatic degradation of the ester bond in BEN molecule, it was suggested to store collected samples immediately in the dark at −40 °C [9]. In the other papers there were no evidence of the light sensitivity, and the long-term stability of both the drug and the metabolite at −20 °C was confirmed. The peak plasma concentration after administration of 10 mg capsule to the healthy volunteer was reported to be ca. 220 ng/mL for BEN and ca. 330 ng/mL for BEN-at, respectively [9].

4.2. Captopril (CAP)

To prevent the formation of disulphide dimers or other conjugates of CAP, chemical stabilization was required. Two reagents were used for this purpose: NEM for GC methods and 1,4-dithio-DL-threitol for LC assays. The determination of CAP in biological fluids was more often performed using gas chromatography [11–16,18] than liquid chromatography [17]. In the papers from the early 1980s packed columns were used, while the recent works applied more efficient capillary columns. The advances in separation technique improved LLOQ from 20.0 ng/mL [14] to 0.5 ng/mL [16]. The LC–MS/MS method, whose LLOQ was determined at 25 ng/mL, was still sensitive enough to be applied to the single dose pharmacokinetic study of CAP 50 mg tablets (observed mean maximal concentration was ca. 1800 ng/mL) [17]. Generally, the positive ionization was employed, however the lowest LLOQ (0.5 ng/mL) was obtained with the negative ionization [16]. This method allowed the determination of lower concentrations of CAP after a 25 mg dose (C_{max} ca. 180 ng/mL) [17]. The CAP was reported to be a stable compound, with no significant degradation after storing human plasma for 16 h at r.t., nor for 99 days at −20 °C. The stability of CAP solutions stored in a refrigerator at 4 °C for 3 months was also confirmed.

4.3. Delapril (DEL)

DEL is extensively metabolized by esterases to the diacid derivative (DEL-at), which – in turn – is converted to 5-hydroxy-delaprilat (M3) and an inactive diketopiperazine monoacid (M2). DEL is pharmacologically active but both DEL-at and M3 are more potent ACE inhibitors [2]. A single paper on hyphenated chromatographic determination of DEL and its metabolites was found [19]. The work reported the results of the pharmacokinetic interaction study of DEL and manidipine, a calcium channel antagonist. The tandem mass spectrometry method made it possible to quantify simultaneously DEL, DEL-at and two other metabolites after simple SPE on C18 cartridges. The absolute extraction yield from plasma was about 80% for all compounds. The method involved positive ionization, but no data on the monitored ions was enclosed. The investigation of stability of DEL and its metabolites showed no significant degradation after 6 months plasma storage at −20 °C. The extracted samples were stable for 21 h at r.t. There was no degradation in plasma stored at r.t. neither during 4 h nor after three freeze–thaw cycles. The method allowed to determine the concentration of DEL and its three metabolites after a single administration of Delapril 30 mg tablet. The peak concentration was 221 ng/mL for DEL, 477 ng/mL for DEL-at, 157 ng/mL for M2 and 171 ng/mL for M3, respectively.

4.4. Enalapril (ENA)

Liquid chromatography–mass spectrometry methods facilitated the simultaneous determination of ENA and ENA-at in human plasma samples [20,22,23]. The GC–MS method described the quantification of the active metabolite [21]. In contrast to tandem mass spectrometry methods, the single LC–MS assay included a derivatization step during sample pretreatment [23]. The authors declared that the treatment with diazomethane resulted in an increased sensitivity and reproducibility. The most sensitive of the described methods employed the simplest sample preparation technique (LLE), but the extraction recovery of ENA-at was very low (22–26%) [22]. The analysis of the human plasma samples from 20 healthy subjects after a single 10 mg oral administration provided the following mean C_{max}: 114 ± 42 ng/mL for ENA and 35 ± 15 ng/mL for ENA-at, respectively [22]. The positive ionization was used in all the above-mentioned methods. The smallest LLOQ (0.1 ng/mL) was obtained in the LC–MS/MS assay with a turbo ion spray source [22], while with the GC–MS method the LLOQ = 5 ng/mL was obtained [21].

4.5. Fosinopril (FOS)

FOS is completely hydrolysed by intestinal and liver esterases to the active metabolite, fosinoprilat (FOS-at). The pharmacokinetic results indicate that after an oral dose of ^{14}C-labelled FOS, about 75% of the radioactivity in plasma and urine was present as FOS-at, while less than 1% of unchanged FOS was present [91]. C_{max} of FOS-at ranged from 131 ng/mL with a 10 mg dose to 5598 ng/mL with a 640 mg oral dose of FOS [91].

Before the application of liquid chromatography coupled with mass spectrometry to FOS determination, alkaline hydrolysis of prodrug to FOS-at was necessary. The LC–MS assay allowed the simultaneous quantification of both prodrug and its active metabolite [25]. The method employed acidification (with the phosphoric acid solution) of the serum samples to minimize the hydrolysis of FOS to FOS-at prior to purification by SPE. In the latter assay, acidification of serum samples was achieved using sodium acetate buffer (pH 4.0) [24]. The authors stressed that in the proposed analysis conditions the conversion of FOS to FOS-at is relatively insignificant (<1%). The fully automated sample preparation performed on 96-well plates, combined with the low amount of sample (0.2 mL) and the short analysis time of 2.0 min, provided impressive efficacy, but only quantification of the active metabolite was possible [24]. In the former method [25], a turbo ion spray source was used, and a slightly better

sensitivity was achieved than in the latter assay with an electrospray ion source. Both methods utilized positive ionization. Both FOS and FOS-at were reported to be stable in plasma after three freeze–thaw cycles and long-term storage at −20 °C for at least 181 days. The post preparative stability was confirmed at r.t. up to 48 h [24].

4.6. Imidapril (IMI)

The GC–MS/MS method was used for determination of three metabolites other than IMI-at [26]. The method was applied to both human plasma and urine samples. Among liquid chromatography methods, tandem mass spectrometry assay enables the quantitation of IMI and IMI-at after protein precipitation and SPE on HLB cartridges [27]. The method was compared to radioimmunoassay and the close correlation of the results was obtained (correlation coefficient $r^2 > 0.81$). The single LC–MS assay with a simplified sample pretreatment was nearly as sensitive as the tandem one, but did not determine IMI-at [28]. The sensitivity of LC–MS methods was 10 times higher than in the GC–MS/MS method. The determinations were performed with the positive ionization. The stability of IMI and IMI-at in plasma samples was investigated at −20 °C [28]. Both compounds did not show significant degradation during long-term (10 days), nor three freeze–thaw cycles. The plasma level of IMI in eight healthy subjects after administration of 10 mg reached maximum at 35 ± 10 ng/mL [28]. The same dose administered to four healthy volunteers lead to C_{max} values of ca. 28 ng/mL and 13 ng/mL for IMI and IMI-at, respectively [26]. The latter values were determined by HPLC method with fluorimetric detection.

4.7. Lisinopril (LIS)

Two GC–MS negative ion chemical ionization methods were introduced for the determination of LIS in human plasma [29,47]. In both methods derivatization procedures were used. In the first method LIS was derivatized with the methyl diester trifluoroacetamide. Due to the interferences from the matrix plasma, the derivatization procedure was changed. It involved derivatization of LIS, extracted by SPE on C18 sorbent, to its pentafluorobenzyl diester trifluoroacetamide derivatives [29]. This method was highly sensitive (LLOQ = 0.4 ng/mL) and selective enough to determine the therapeutic plasma levels of LIS, although the operation procedures prior to the analyses seem to be complicated. LC–MS–(MS) assays of LIS had the advantage of sample introduction without derivatization. The analytes were extracted from acidified plasma by liquid–liquid [35], solid-phase extraction [30–32,34] and deproteinization with trifluoroacetic acid [33]. The recoveries observed were in the range of 79–96% [30–32,34]. However, the liquid–liquid extraction yield was about 42% [35]. The positive ESI mode was used for LIS detection. No evidence of matrix effect was observed [32,35]. The following compounds were used as internal standards: ENA, ENA-at, pseudoephedrine hydrochloride and ciprofloxacin. Typical LLOQ obtained by means of LC–MS–(MS) methods ranged from 0.78 ng/mL to 10 ng/mL. Mean maximum serum concentrations of LIS were ca. 40 ng/mL after a single oral dose of 10 mg and about 80–140 ng/mL after a 20 mg dose. Plasma serum was detectable for 72–96 h after 10 or 20 mg dose [92]. Plasma LIS was found to be stable at −20 °C for at least 2 weeks [30], 3.5 months [32] and 5 months [34].

4.8. Moexipril (MOE)

A single paper reporting the development of a hyphenated method for the simultaneous determination of MOE and its active metabolite MOE-at in human plasma was retrieved [36]. This GC–MS method used QUI and QUI-at as the internal standards. The process of sample preparation included four steps. Firstly, the sample clean up on SPE C18 cartridges was applied. The derivatization step, methylation with diazomethane, was followed by acid–base partition as a purification step and, finally, trifluoroacetamidation was performed. This method was sensitive enough to appoint LLOQ at 0.5 ng/mL level and was used for various pharmacokinetic studies in human subjects. The peak plasma concentrations for MOE and MOE-at after oral administration of 15 mg dose to human subject were ca. 53 ng/mL and 27 ng/mL, respectively. The presented concentration–time curve plot suggests that described method is suitable for the pharmacokinetic studies. The assay utilized negative ion chemical ionization, which was rarely used to detect the compounds in question.

4.9. Perindopril (PER)

A high throughput method, based on the solid phase extraction and LC–MS/MS, was found for the determination of PER and PER-at in human plasma with RAM as I.S. [37]. The assay was very sensitive, with respective LLOQs at 0.5 ng/mL and 0.3 ng/mL. The acidified plasma with concentrated *o*-phosphoric acid prior to solid phase extraction step on HLB cartridges, improved the method ruggedness. The pre-treatment of HLB cartridges with 2% acetic acid reduced the basic matrix retention. The SPE eluate was directly injected onto LC–MS/MS system. Thus, the evaporation and reconstitution steps were omitted, which improved the speed of the assay. In the method, a turbo ion spray source at negative ionization mode was used. The stability of PER and PER-at in plasma was investigated in the following conditions: at room temperature (6 h), during storage at −20 °C (222 days) and during three freeze–thaw cycles. The results indicate that both drug and the metabolite are stable compounds. Additionally, the stability of an extract at 10 °C up to 48 h, and the stock solution at r.t. for 24 h was confirmed. The administration of a single dose of 4 mg to healthy volunteers lead to peak plasma concentrations of ca. 135 ng/mL and 14 ng/mL for PER and PER-at, respectively.

4.10. Quinapril (QUI)

Two papers on GC–MS assays of QUI and its metabolites reported complementary methods, which allowed for the detailed study of QUI pharmacokinetics. The first work reported

the concurrent determination of QUI and QUI-at with the negative ionization [38], with both LLOQ values at 0.05 ng/mL for plasma and at 0.5 ng/mL for urine. The second paper, which dealt with positive ionization, reported the quantification of two dioxopiperazine metabolites [39]. Both procedures were applied to human plasma and urine samples. A single LC–MS method for determination of QUI and QUI-at in human plasma was developed [40]. After SPE on C18 cartridges, elution with dichloromethane–MeOH (2:1), evaporation and reconstitution of samples in the mobile phase, the MS analysis was performed. In the assay an electrospray source with positive ionization was applied. The LLOQ values were 5 and 10 ng/mL for QUI and QUI-at, respectively. Both the drug and the metabolite were stable in plasma for 4 months at −20 °C, while the stock solutions stability at −20 °C was confirmed for at least 1 month [65]. After a single dose of 40 mg to a healthy volunteer the C_{max} of ca. 850 ng/mL and 550 ng/mL were reported for QUI and QUI-at, respectively [65].

4.11. Ramipril (RAM)

The RAM determination assays best demonstrate the development of ACEIs quantification in the biological material. The first method developed in the 1980s was radioimmunoassay with the lower limit of quantification of 0.5 ng/mL [56]. Then, the voltammetric (LLOQ = 160 ng/mL) and spectrofluorometric (LLOQ = 20 ng/mL) methods with simple LLE were proposed [73,77]. Application of hyphenated chromatographic techniques improved the sensitivity in comparison to radioimmunoassay. The LLOQ of GC–MS method, including SPE and derivatization steps during the sample pretreatment, was determined at 0.4 ng/mL [42]. The LC–MS/MS method was even more sensitive (LLOQ = 0.1 ng/mL) and, furthermore, the protein precipitation with MeOH was the simplest sample preparation applied to RAM determination [43].

All three overviewed RAM and RAM-at assays were very sensitive. LC–MS methods allowed to measure the prodrug and active metabolite concentrations in plasma/serum samples [41,43], while GC–MS method also allowed the quantitative analysis in urine [42]. Zhu et al. observed *cis–trans* isomerization associated with peak splitting [41]. To improve the peak shape, the authors proposed the use of both organic modifiers (MeOH and ACN) in the mobile phase and elevated oven temperature (55 °C). The gas chromatography method was designed to eliminate the interference from glucuronide conjugates, which was achieved by applying a washing step between alkylation and acetylation. The plasma stability of RAM and RAM-at was investigated in the following conditions: 24 h at r.t. [43], three freeze–thaw cycles and 43 weeks at −20 °C [41]. The obtained results indicated that no significant degradation occurred. The recoveries of RAM and RAM-at from serum were in the range of 81–98%. The LC–MS method was applied for the determination of pharmacokinetic parameters of RAM and RAM-at after single 10 mg dose in 18 Chinese healthy male volunteers [43]. The mean values of the plasma maximum concentration were 42 ± 13 ng/mL for RAM and 43 ± 14 ng/mL for RAM-at.

4.12. Spirapril (SPI)

The GC–MS assay was reported for concurrent determination of a prodrug and its active metabolite in the wide range of concentrations (2.5–500.0 ng/mL) [44]. ENA was used as the internal standard. No interference from the co-administered diuretic hydrochlorothiazide was reported. The sample pretreatment included SPE on C18 cartridges (elution with a mixture of ethyl acetate–MeOH, 3:2) followed by derivatization with TMSDM. There was no information about the fragmentation of the SPI, SPI-at and ENA molecules in the paper, as well as about the ionization source. However, the detailed method validation data obtained for the SPI and SPI-at was presented. The complete stability studies indicate, that SPI and SPI-at are stable in plasma: for 24 h at r.t., after three freeze–thaw cycles and after 12 weeks of storage at −20 °C. The data on real samples concentrations, obtained from the drug interaction study of SPI and hydrochlorothiazide, suggest that the assay described above is sensitive enough for pharmacokinetic investigations [93]. After single oral administration of 6 mg dose, the mean C_{max} of the reference drug was 210 ng/mL and 102 ng/mL, for SPI and SPI-at, respectively.

4.13. Temocapril (TEM)

A single paper reporting hyphenated technique to TEM determination was found [45]. The described GC–MS method facilitated the evaluation of TEM and TEM-at pharmacokinetics and pharmacodynamics, including the analyses of human plasma and urine. The sample preparation method was complicated. Firstly, SPE on C18 cartridges was followed by derivatization with diazomethane. Then, the silica chromatography column was applied and, finally, the derivatization with TFA was performed. The internal standard was a deuterium-labelled TEM-at, which is not available commercially. In this method, negative ion chemical ionization was used. The fragmentation pathways of the TEM, TEM-at and I.S. were presented. The graph of mean plasma concentrations after oral administration of 5 mg dose to healthy volunteers shows C_{max} ca. 25 ng/mL for TEM and ca. 150 ng/mL for TEM-at, respectively. The pharmacokinetic study in young and elderly patients (two groups of 18) showed similar concentrations after a single 20 mg dose and in the steady state, reached after 1 week [94]. The mean values of the plasma maximum concentration after a single dose in both groups ranged 136–180 ng/mL for TEM and 760–863 ng/mL for TEM-at, respectively.

4.14. Zofenopril (ZOF)

The LC–MS/MS method was developed for the determination of prodrug and its active metabolite [46]. To prevent the oxidative degradation of free sulfhydryl groups of ZOF and ZOF-at, plasma samples were treated with NEM. The simple sample pretreatment (LLE with toluene, after the addition of phosphoric acid (6 M) and 2% tetrabutylammonium hydrogen sulphate) and the wide range of linearity (1–300 ng/mL for ZOF and 2–600 ng/mL for ZOF-at) were the main advantages of this

method. The 4-fluoro-phenyl derivatives of ZOF and ZOF-at were used as the internal standards, respectively. In this method, a turbo ion spray source and negative ionization was applied. The extraction recovery was reported to be 85% for ZOF and 70% for ZOF-at, respectively. The stability of both parent drug and metabolite in human plasma after 24 h at r.t and three freeze–thaw cycles was confirmed. The mean peak plasma concentration reported after a single oral 60 mg dose to 20 healthy volunteers was 106 ± 49 ng/mL for ZOF and 767 ± 190 ng/mL for ZOF-at, while after a single oral 30 mg dose the respective C_{max} was 67 ± 16 ng/mL for ZOF and 341 ± 109 ng/mL for ZOF-at [95].

5. Simultaneous determination of ACE inhibitors

The treatment of hypertension is based on a single ACEI administration, thus the concurrent determination of ACEIs in the biological material is of little interest from the pharmacological point of view. Nevertheless, a single method for simultaneous determination of ACEIs was found in the literature [96]. This GC–MS assay in urine was developed as a screening procedure for toxicological purposes and made it possible to analyze BEN, ENA, PER, QUI, RAM, TRA and their metabolites.

6. Conclusions

It should be stressed that almost every method involves the simultaneous measurement of parent prodrugs and their active metabolites (CAP and LIS, which are active as such, being the exception). Therefore, some valuable pharmacokinetic information can be derived after a single analysis of a biofluid sample.

Some of the reviewed methods make it possible to quantify simultaneously ACEIs and diuretics (e.g. hydrochlorothiazide [10,20]) or calcium channel blockers (e.g. manidipine [19]). This kind of assays, which are of practical importance (e.g. bioequivalence studies of combined preparations), were also developed for non-hyphenated HPLC techniques.

To conclude, among various techniques used for the determination of ACEIs in the biological material, the hyphenated methods seem to be very useful. Although still being developed, the GC–MS methods recently seem to be less attractive than LC–MS assays due to a complicated sample pretreatment process. The main advantages of liquid chromatography coupled with mass spectrometry, i.e. selectivity, so important in the analysis of the biological material as well as sensitivity, serve as a strong argument for the possibility of more future applications in the overviewed field.

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