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Review

Analytical methodologies for the determination of omeprazole: An overview

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Abstract

Omeprazole, a gastric acid pump inhibitor, dose-dependently controls gastric acid secretion; the drug has greater antisecretory activity than histamine H_2-receptor antagonists.

Omeprazole has been determined in formulations and biological fluids by a variety of methods such as spectrophotometry, high-performance liquid chromatography with ultraviolet detection and liquid chromatography coupled with tandem mass spectrometry. The overview includes the most relevant analytical methodologies used in its determination since the origin still today.

Keywords: Omeprazole; Pharmaceutical analysis; Review; Analytical methodologies

Contents

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1. Introduction

Omeprazole (OMZ), 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulphinyl]-1*H*-benzimidazole (Fig. 1), a substituted benzimidazole compound and prototype anti-secretary agent, is the first of the "proton pump inhibitors" widely used for the prophylaxis and treatment of gastro-duodenal ulcers and for the treatment of symptomatic gastro-oesophageal reflux. It is a lipophilic, weak base with $pK_{a1} = 4.2$ and $pK_{a2} = 9$ and will be degraded unless it may be protected against acid conditions. It acts by interaction with H^+/K^+ ATPase in the secretory membranes of the parietal cells and is very effective in the treatment of Zollinger–Ellison syndrome. Although its elimination half-life from plasma is short, reported to be about 0.5–3 h, its duration of action with regard to inhibition of acid secretion is much longer allowing it to be used in single daily dose.

OMZ contains a tricoordinated sulphur atom in a pyramidal structure and therefore can exist in two different optically active forms, (*S*)- and (*R*)-omeprazole. OMZ was first approved as a racemic mixture, but the (*S*) isomer was recently introduced on the market. Both enantiomers have a similar inhibitory effect on acid formation in isolated gastric glands from rabbits, but (*R*)-omeprazole is stereoselectivelly hydroxylated by cytochrome P450 CYP2C19 enzyme, resulting in an almost two-fold increase in the plasma concentration for the (*S*)-isomer than for racemic OMZ after the administration of equivalent doses.

OMZ is metabolized principally by CYP2C19 to generate 5′-hydroxy-omeprazole (OH-OMZ), and a minor pathway, through CYP3A4 enzymes generates omeprazole-sulphone (OMZ-SO_2).

The development of new methods capable of determining drug concentration in pharmaceutical formulations and biological samples is important.

2. Spectrophotometric methods

2.1. OMZ

OMZ has been assayed by the A_{max} method directly [1]. The A_{max} method has been proved to be inaccurate due to matrix interference. In view of the fact that there is no visible spectrophotometric method for the determination of OMZ, sensitive and accurate visible spectrophotometric methods were viewed as essential to avoid interference due to UV absorbing compounds in the determination of OMZ in bulk samples or pharmaceutical dosage forms.

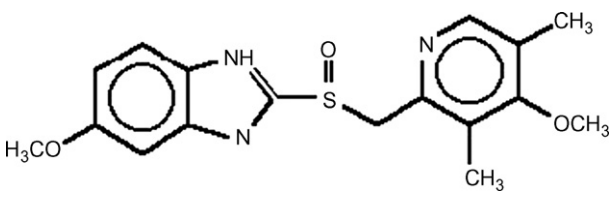

Fig. 1. Chemical structure of omeprazole.

In this way, Sastry et al. [2] describe four simple and sensitive methods, based on the formation of coloured compound with

- Method A: 3-methyl-2-benzothiazolinone hydrazone following oxidation with ferric chloride (linear range 1.0–10 mg L^{-1}).
- Method B: *m*-aminophenol following oxidation with chloramine-T (linear range 2.0–32 mg L^{-1}).
- Method C: Folin Ciocalteau reagent in the presence of sodium carbonate (linear range 0.4–2.4 mg L^{-1}).
- Method D: by oxidizing OMZ with excess *N*-bromosuccinimide and determining the consumed reagent with a decrease in colour intensity of Celestine blue (0.8–10 mg L^{-1}).

The order of sensitivity among the proposed methods and UV reference method (*R*) in the determination of OMZ is D > C > A > R > B. The λ_{max} order is C > A > D > B > R. Although the molar absorptivity of one of our methods (B) was some what less than that of the reference method, the λ_{max} of all the four proposed methods were considerably higher than that of the reference method. The higher λ_{max} of the proposed methods is a decisive advantage since the interference from the associated ingredients shall be far less at higher wavelengths. The validity of each method was tested by analysing OMZ in capsules.

Also, flow-through spectrophotometric technique was used by Tuncel and Dogrukol for the determination of OMZ in pharmaceutical preparations containing enteric coated pellets [3].

In the last years, derivative techniques in UV spectrophotometry have been used as separative methods for the analysis of different commercial preparations as well as in stability studies. Ozaltin and Kocer [4] use derivative UV spectroscopy for the analysis of OMZ in borate buffer of pH 10.0; second derivative spectra were generated between 200 and 400 nm (linear range 0.2–40 mg L^{-1}).

A first derivative spectrophotometric method was developed for the determination of OMZ in aqueous solution by measuring the derivative amplitude at 313 nm. The method has been described to be 2.59 times more sensitive than the official HPLC method [5]. OMZ has also been determined in presence of its photo-degradation product (sulphenamide and benzimidazole sulphide) by derivative spectrophotometry (^1D, ^2D and ^3D) and complex formation [6]. The first method depends on use of first, second and third derivative spectrophotometry at 290.4, 320.6 and 311.6 nm, respectively. The second method is based

on applying the charge-transfer technique with chloranil as π receptor to form a complex with OMZ, the absorbance of which is measured at 377 nm.

From the different methods studied, direct spectrophotometry and second derivative spectrophotometry were highly influenced by the decomposition products of OMZ and were not suitable for the quantitation of OMZ in stability studies. However, the first derivative procedure was successfully applied to quantify OMZ in such studies.

In other study, the objective is to investigate the degradation of OMZ in organic polymer solutions and aqueous dispersions of enteric coating polymers by UV spectroscopy [7]. The discoloration of degraded OMZ solutions was analysed. UV–vis spectra were recorded after preparation of the solutions and after 180 min of storage. The change of absorption was calculated as the difference of the absorption values at 305 nm.

2.2. Mixtures

2.2.1. Omeprazole and domperidone

Lakshmi et al. [8] utilize a Shimadzu UV-1601 instrument and the measurement were carried out at λ_{max} 272 and 286 nm for omeprazole and domperidone, respectively, using methanol as solvent (linear ranges 10–60 and 5–30 mcg, respectively). The method was applied in combined capsule dosage form.

2.2.2. Omeprazole (OMZ) and pantoprazole (PNZ)

Both drugs are descomposed in acid media to yield two main products sulphonamide and sulphenic acid. OMZ was also found to be unstable in neutral and weak alkaline media where its maximum stability was at pH 11. Salama et al. [9] propose procedures based on the formation of 2:1 chelates of both drugs with different metal ions. The coloured chelates of OMZ with Fe(III), Cr(III) and Co(II) in ethanol are determined spectrophotometrically (linear ranges 15–95, 10–60 and 15–150 mg L^{-1}, respectively).

Karljikovic-Rajic et al. [10] use first UV derivative spectrophotometry, applying zero-crossing method for the determination of OMZ, omeprazole-sulphone (OMZS), PNZ, and *N*-methylpantoprazole (NPNZ) in methanol–ammonia 4.0% (v/v), where the sufficient spectra resolutions of drug and corresponding impurity were obtained, using the amplitudes $^1D_{304}$, $^1D_{307}$, $^1D_{291.5}$ and $^1D_{296.5}$, respectively. The results obtained showed that the proposed zero-crossing method in the first-order derivative spectrophotometry, applying the method of standard additions, could be used as stability-indicating method of OMZ and PNZ. The achieved results also confirmed that derivative spectrophotometry, using zero-crossing method, could be used to analyse possible pH induced impurity–drug or drug–drug interactions.

2.2.3. OMZ, lansoprazole (LNZ) and PNZ

Different colorimetric methods have been described for the determination of the three drugs in their single component dosage forms based on their reactions with various reagents. Both OMZ and LNZ have been analysed in their enteric coated granules using the second derivative spectrophotometric method. A two-wavelength spectrophotometric method by measuring the peak-trough amplitude at 293 and 320 nm has also been described [11]. Wahbi et al. [12] applied the compensation method and other chemometric methods (derivative, orthogonal function and difference spectrophotometry) for the direct determination in pharmaceutical preparations. The difference spectrophotometric method is unaffected by the presence of acid induced degradation products; hence can be used as a stability indicating assay. The disadvantage of the proposed methods is that they cannot be applied to biological fluids containing these compounds and their conjugated forms.

In the same way, four stability indicating assays were developed by El-Kousy and Bebawy [6] for determining omeprazole and octylonium bromide.

Analytical data for some methods described in the text find in Table 1.

3. Electrochemical methods

Electroanalytical techniques have been used for the determination of a wide range of pharmaceuticals with the advantages that there is in most instances, no need for derivatization, and that these methods are less sensitive to matrix effects than other analytical techniques.

3.1. OMZ

Various polarographic techniques and supporting electrolytes were examined by Dogrukol and Tuncel [13] and Özaltin and Temizer [14]. These authors reported that the best results were obtained with differential pulse polarography (DPP) in borate buffer (pH 9.0) solution. The peak potential was observed at −1.28 V (versus Ag/AgCl). DPP is also employed by Knoth and co-workers [15,16] for the determination in Britton–Robinson (B–R) buffers (pH 7–9) up to a concentration of 10^{-5} M. Recently, Qaisi et al. investigated the reactions of OMZ in the absence of a nucleophile. Reactions were monitored, using DPP at the static mercury drop electrode, in solutions buffered to pH values ranging from 2.0 to 8.0. Fast recording of each polarogram (153 s) facilitated the evaluation of current–time profiles of all species in situ [17].

The adsorptive stripping voltammetric (ASV) method, used by Pinzauti et al. [18], was set up using a multivariate strategy by means of experimental design tools. The optimized method shows a good linearity between peak height and analyte concentration in the concentration range from 8.33×10^{-9} to 1.42×10^{-7} M with a detection limit of 6.5×10^{-9} M. The mean recovery of OMZ in capsules was 101.9%.

Yan [19] use glass carbon electrode as working electrode for the determination of OMZ for the first time. In HAc/NaAc buffer solution, pH 5.10, a sensitive differential pulse voltammetric (DPV) peak at about +0.74 V is found. The electrochemical behaviour and reaction mechanism of this system have been studied by cyclic voltammetry, linear sweep voltammetry and differential pulse voltammetry.

Table 1
Spectrophotometric methods: analytical data

Method	Analytical data		Samples	Reference
	λ_{max} or range (nm)	DL[a] or linear range ($\mu g\,mL^{-1}$)		
3-Methyl-2-benzothiazolinone hydrazone following oxidation with ferric chloride	660	0.074	Capsules	[2]
m-Aminophenol following oxidation with chloramine-T	420	0.104		[2]
Folin Ciocalteau reagent in the presence of sodium carbonate	540	0.023		[2]
By oxidizing OMZ with excess *N*-bromosuccinimide and determining the consumed reagent with a decrease in colour intensity of Celestine blue	770	0.039		[2]
Second derivative; in borate buffer (pH 10.0; 0.1 M)	303–310	0.2 for signal-to-noise ratio of 8.1	Five different commercial preparations of hard gelatine capsules containing enteric coated granules	[4]
First derivative	313	0.49	In aqueous solutions during stability studies	[5]
First, second and third derivative	290.4, 320.6, 311.6, respectively	5–20	Bulk powder, laboratory-prepared mixtures, and pharmaceutical dosage forms	[6]
Applying the charge-transfer technique with chloranil as π acceptor to form a complex with OMZ	377	10–50		[6]
Coloured chelates of OMZ in ethanol with Fe(III), Cr(III) and Co(II)	411, 339, 523, respectively	0.70, 1.98, 0.22, respectively	Dosage forms	[9]
First derivative	304	1.126	Analysis of impurities–degradation products in the presence of their parent drug	[10]
Second derivative	306.2	2–42	Gastro-resistant formulations	[12]
Orthogonal function	306	5–35		[12]
ΔA (intact vs. degradation in NaOH)	256	5–40		[12]
ΔA (intact vs. degradation in HCl)	280	7–40		[12]

[a] Detection limit.

3.2. Mixtures

3.2.1. OMZ and ATPase inhibitors

DPP has been used to monitor the degradation of benzimidazole sulphoxide antiulcer drugs SK&F 95601 and OMZ in 10^{-2} mol L^{-1} HCl to two main products, i.e., the sulphenamide and the benzimidazole sulphide. It is also used to follow the reactions of 2-mercaptoethanol with the respective products of these degradations, as simulations of their believed reactions in vivo. These reactions are also followed by UV spectrophotometry, spectrofluorimetry and liquid chromatography [20].

3.2.2. OMZ and LNZ

Belal et al. [21] employed anodic polarographic in B–R buffer over the pH range 4.1–11.5. At pH 7 well-defined anodic waves were produced (linear ranges 2–16 and 4–24 mg L^{-1} for OMZ and LNZ, respectively, by using Direct Current mode; 0.4–12 and 2–18 mg L^{-1} for OMZ and LNZ, respectively, by using DPP mode).

The reduction process and quantitative determination of OMZ have been studied by means of several polarographic techniques [13–17], but up to now nothing has been published concerning electrochemical oxidation of lansoprazole or omeprazole at solid electrodes. The oxidation behaviour of

Table 2
Electrochemical methods

Method	DL[a] or/and linear range	Samples	Reference
DPP	2.0×10^{-7} to 2.0×10^{-5} M	Two different commercial hard gelatine capsules, containing enteric-coated granules, preparations	[14]
ASV	6.5×10^{-9} M; 8.33×10^{-9} to 1.42×10^{-7} M	Capsules	[18]
DPV	0.19 mg L^{-1}; 1–20 mg L^{-1}	Enteric-coated tablets	[19]
DPP	0.8×10^{-7} M; $(1–10) \times 10^{-7}$ M	In formulation and body fluid	[20]
DPP	1.45×10^{-7} M	Commercial capsules	[21]
DPV	2.5×10^{-8} M	Capsules	[22]

[a] Detection limit.

these drugs on carbon paste electrode is described by Radi and voltammetric method was developed for quantitative determination of OMZ or LNZ in capsules [22]. By differential-pulse voltammetry, a linear response was obtained in B–R buffer pH 6.0 in a concentration range from 2.0×10^{-7} to 5.0×10^{-5} M for the two drugs. The detection limits were 1.0×10^{-8} and 2.5×10^{-8} M for LNZ and OMZ, respectively.

Table 2 shows the detection limits of OMZ obtained by electrochemical methods.

4. High-performance liquid chromatography (HPLC)

OMZ is extensively metabolized by the liver to several metabolites, omeprazole-sulphone, 5-hydroxyomeprazole and omeprazole sulphide. Sulphone and hydroxy are the major metabolites found in plasma and hydroxy is the predominant one in urine. The concentration of sulphide is usually too low to be determined in plasma, and sulphide or OMZ is also negligible in urine. Because OMZ is metabolized extensively by the hepatic cytochrome P450 (CYP) 2C19 and exhibits wide interindividual variability in plasma concentration, its metabolic profile has been investigated in the evaluation of metabolic activity related to CYP2C19. Several HPLC methods have been available for the determination of OMZ and its metabolites in biological fluids.

4.1. UV detection

Mihaly et al. describe the first assay for the determination of OMZ in human plasma and urine, which is also suitable for the simultaneous measurement of the sulphone and sulphide metabolites, using a selective reversed-phase HPLC method with a sensitivity of 5 ng mL^{-1} for omeprazole, 30 ng mL^{-1} for omeprazole-sulphone, and 50 ng mL^{-1} for omeprazole sulphide [23]. Although these sensitivities, resolution of these components, the peak sharpness (widths) and the plasma extract front seen in the chromatograms did not appear to be supportive of the adequate quality control necessary at these levels. In addition, the coefficients of variation for within-day and day-to-day assays were only reported for concentrations well above their sensitivity values. Another method utilizing normal-phase liquid chromatography and UV detection reported sensitivities for OMZ at 10 ng mL^{-1} when analysed alone or in conjunction with the sulphone metabolite [24], however, sensitivities declined by two- to three-fold upon analysis which also included the sulphide metabolite. Amantea and Narang report a method for simultaneous determination of OMZ and its two metabolites that has been significantly modified to achieve greater sensitivity with better reproducibility and accuracy [25].

After, Lagerstrom and Persson [26] describe methods for the determination of OMZ and three of its metabolites, sulphone and sulphide in plasma, and the hydroxy metabolite in plasma and urine. The methods comprise extraction from plasma into methylene chloride followed either by direct injection of part of the organic extract onto a normal-phase liquid chromatography column or, for the more polar hydroxy metabolite, evaporation of the organic extract, dissolution into an aqueous phase and injection onto a reversed-phase column. The compounds are detected by an ultraviolet monitor.

Two manual methods for the determination of OMZ in biological fluids have been published previously [24,26]. In order to provide the large number of plasma and urine assays required for the documentation of the drug, an automated method is desirable. High sample throughput is achieved in a first study by application of the Technicon Fully-Automated-Sample-Treatment-LC technique to the determination in plasma and urine of OMZ and two metabolites hydroxy and sulphone. Sample preparation is achieved by an air-segmented continuous-flow system providing solvent extraction, evaporation to dryness and reconstitution before injection onto a reversed-phase column. The compounds are separated by isocratic or gradient elution with acetonitrile phosphate buffer mobile phases and quantified by UV-measurements at 302 nm [27]. Sensitive and reliable HPLC method was validated for the simultaneous measurement of OMZ and 5-hydroxyomeprazole (5OH-OMZ) in human plasma by Tata and Bramer [28]. OMZ, 5OH-OMZ, and OPC-18827 (the internal standard) were extracted from human plasma by liquid–liquid extraction into CH_2Cl_2–isopropanol (9:1). Analytes were resolved using a C-18 HPLC column and gradient elution mobile phase containing 50 mM phosphate buffer in acetonitrile (22–50% in 43 min followed by 15 min equilibration). The eluents were monitored by UV detection at 302 nm.

Recently, Zarghi et al. [29] using a monolithic column for quantification of OMZ in plasma. The separation was carried out in reverse-phase conditions using a Chromolith Performance (RP-18e, 100 nm × 4.6 mm) column with an isocratic mobile phase consisting of 0.01 M disodium hydrogen phosphate buffer–acetonitrile (73:27) adjusted to pH 7.1 (detection at 302 nm).

Several HPLC methods have been available for the determination of OMZ and its metabolites in biological fluids. However, only one of the reversed-phase HPLC methods reported previously allows a simultaneous determination of OMZ and its major metabolites, sulphone and hydroxy, in plasma [27]. Although two reversed phase HPLC methods have enabled OMZ, sulphone and sulphide to be determined in plasma [25] and in plasma and urine [23], these methods do not determine hydroxy in human biological fluids. Although an HPLC method has been used to determine OMZ, sulphone and hydroxy in plasma and hydroxy in urine [26], it involves two separate HPLC systems: normal-phase for OMZ and sulphone in plasma and reversed-phase for hydroxy in plasma and urine. Kobayashi et al. report a method for the determination of OMZ, sulphone and hydroxyl in plasma simultaneously and hydroxy in urine by using a conventional HPLC system with an alkaline-resistant column packed with polymer-coated C_{18} packing material. The method was applied to a preliminary pharmacokinetic study of OMZ and its metabolites in healthy volunteers [30]. In this way, Macek et al. describe a method for determination of OMZ in plasma using an alternative solvent toluene-isoamylalcohol which yields cleaner extracts and flunitrazepam as an internal standard [31], Gangadhar et al. measured OMZ in human plasma using a Zorbax C_8 column with a mobile phase of acetonitrile:phosphate buffer of pH 7.5 (detection limit in plasma was 1 ng mL^{-1}) [32] and

Yuen et al. [33] developed a method where OMZ and the internal standard, chloramphenicol, were extracted from alkalinized plasma samples using dichloromethane. The mobile phase was 0.05 M Na_2HPO_4:acetonitrile (65:35) adjusted to pH 6.5. Analysis was run at a flow rate of 1.0 mL min^{-1} at a detection wavelength of 302 nm. The method was specific and sensitive with a detection limit of 2.5 ng mL^{-1} at a signal-to-noise ratio of 4:1. The limit of quantification was set at 5 ng mL^{-1}.

Because OMZ is metabolized extensively by the hepatic cytochrome P450 (CYP) 2C19 and exhibits wide inter-individual variability in plasma concentration, its metabolic profile has been investigated in the evaluation of metabolic activity related to CYP 2C19. Yim et al. [34] employed a column-switching system with semi-micro-columns for direct analysis of OMZ and sulphone in human plasma samples. The primary separation of proteins and other non-specific peak producing substances was performed with a mixed-function column (Capcell Pak MF Ph-1). The analyte-containing fraction was thereafter transferred to a C_{18} semi-micro-column after concentration at the C_{18} intermediate column. The absorbance was measured at 302 nm (detection limit was 10 ng mL^{-1} for two analytes). In the similar way, González et al. [35] present a method where OMZ, hydroxyl and sulphone were extracted from plasma samples with phosphate buffer and dichloromethane-ether (95:5). HPLC separation was achieved using an Ultrasphere ODS C_{18} column. This method can be also used for studying CYP2C19 and CYP3A4 genetic polymorphisms using OMZ as the probe drug.

Recently, Shimizu et al. [36] developed a method for the simultaneous determination of OMZ and its two main metabolites in human plasma: OMZ, its two metabolites and lansoprazol as an internal standard were extracted from 1 mL of alkalinized plasma sample using diethyl ether–dichloromethane (45:55). The extract was injected into a column I (TSK-PW precolumn, 10 μm, 35 mm × 4.6 mm i.d.) for clean-up and column II (Inertsil ODS-80A column, 5 μm, 150 mm × 4.6 mm i.d.) for separation. This method was suitable for use in pharmacokinetic studies in human volunteers, and provides a useful tool for measuring CYP2C19 activity.

Also, recently, Rezk et al. [37] developed an assay for the simultaneous quantitative determination of OMZ and its three metabolites in human plasma. This method provides excellent chromatographic resolution and peak shape for the four components and the internal standard within a 17 min run time. Simplicity and high throughput make this method suitable for clinical pharmacokinetic studies.

As can be see, several HPLC-UV methods for the determination of OMZ in biological fluids have been developed, but all of these methods involve liquid–liquid extraction, under alkaline condition, which is time-consuming and not economically feasible for routine use in pharmacokinetic studies with numerous samples to be analysed. Jia et al. [38] proposed a method that employs a micro-volume of plasma (150 μL) and a simple sample preparation without organic solvent extraction which ensure the applicability of the method when only small volume of plasma is available. Plasma samples after pre-treatment with acetonitrile to effect deproteinization were dried under N_2 at 40 °C and reconstituted with mobile phase.

Also, liquid–liquid extractions are gradually being replaced by solid phase extraction (SPE) because this latter method is faster, more straight forward, solvent saving, avoids the manipulation of toxic solvents, and is often more reproducible. In this sense, Motevalian et al. [39] developed a rapid, simple and sensitive HPLC assay for the simultaneous determination of OMZ and its major metabolites in human plasma using a SPE procedure.

García-Encina et al. [40] describes the validation of a method to analyse OMZ in human plasma by means of a completely automatic system that uses a solid-phase extraction that is connected on-line with a liquid chromatograph. The extraction was carried out using C_{18} cartridges. After washing, OMZ was eluted from the cartridge with mobile phase onto an Inertsil ODS-2 column.

Dubuc et al. [41] describe an HPLC method for the determination of OMZ in human plasma using a SPE technique that is applied to a diode array detector. The DAD enables detection of two wavelengths simultaneously. OMZ and the internal standard (H168/24) were extracted from plasma samples by SPE using a polymeric sorbent-based cartridge. The separation was accomplished under reversed phase conditions using an Eclipse XDB-C_8 Rapid Resolution column.

On the other hand, 25% of all drugs used as therapeutic agents are chiral compounds administered to humans as racemates, a mixture of enantiomers which may have very different pharmaceutical properties. This fact resulted in an increase in the demand for enantioselective methods for the analysis of drugs in raw materials and pharmaceutical formulations in order to check the enantiomeric purity. HPLC has been widely used for the enantioselective analysis of chiral drugs, mainly by using chiral stationary phases. Over the last two decades a large number of chiral stationary phases have been described in the literature and some of them are commercially available. OMZ is a substituted benzimidazole containing a sulphoxide group and is also administered as a racemate. The two enantiomers of OMZ and related benzimidazoles were separated by Allenmark et al. [42] using a stationary phase of bovine serum albumin (BSA) immobilized on silica. After, Erlandsson et al. [43] used trisphenylcarbamoylcellulose coated on 3-aminopropylsilica, Marle et al. [44] cellulose immobilized on diol silica and VanDenBosch et al. [45] three different protein-based phases in a comparative study. In other study, Balmer et al. [46] examined the separation of the enantiomers of OMZ on different chiral stationary phases and also of three structural analogues on one of the columns. Their used three phases with immobilized protein: chiral-AGP with α_1-acid glycoprotein, Ultron ES-OVM with ovomucoid and BSA-DSC with bovine serum albumin cross-linked into 3-aminopropylsilica using *N*-succinimidyl carbonate. Their separate the enantiomers on all three columns and its studies were limited to the effect of pH and a comparison of two organic modifiers in the aqueous mobile phase. On Chiracel OD and Chiralpak AD, which contain cellulose and amylase tris (3,5-dimethylphenylcarbamate), respectively, coated on macroporous silica their also achieved the resolution of the enantiomers of OMZ. The best enantio-selectivity

was achieved with Chiralpak AD, where interesting selectivity effects were observed by variation of the nature of the alcohol as modifier in the non-polar mobile phase. Also, Tanaka et al. proposed the use of cellulose-based chiral stationary phases in reversed phase mode for separation of enantiomers [47].

Karlsson and Hermansson used a factorial design for optimization of chiral separation of OMZ and hydroxyomeprazole on immobilized α_1-acid glycoprotein, where mobile phase pH, concentration of a mobile phase modifier, ionic strength and column temperature were tested as the variables and enantioselective retention, column efficiency and asymmetry factor as the responses. The system was used for a separation of the enantiomers of OMZ and its main metabolite in a patient plasma sample [48].

On the other hand, although a number of enantiomeric separations for measuring the enantioselective metabolism of OMZ are described in the literature [49–52], the main restriction of those methods are the time spent on sample preparation. Cairns et al. [52] made use of a C_2 solid phase extraction cartridge followed by filtration on a nylon filter for the enantioselective measurement of OMZ using a Chiral AGP column. While, the work of Tybring et al. [49] reports that the enantioselective separation of OMZ was preceded by isolation of the enantiomers using a C_{18} column. Kanazawa et al. [50], in a study of CYP2C19 phenotyping, determined OMZ enantiomers in plasma using a Chiralpak AD-RH column, solid phase extraction SPE on C_{18} cartridges and circular dichroism and UV detection. Also, it was demonstrated that both enantiomers have the same in vitro capacity to decrease gastric acid formation, but stereoselective metabolism by CYP2C19 results in different plasma concentrations [49]. Some authors have already described the resolution of OMZ enantiomers employing chiral columns. A laboratory-made amylase tris-3,5-dimethylphenylcarbamate chiral column was also used by Cass et al. [53] for the resolution of OMZ enantiomers. Bonato et al. [54] evaluated several chiral stationary phases based on polysaccharide derivatives and proteins. Orlando and Bonato utilized C_{18} SPE cartridges to extract the enantiomers form plasma samples and the chiral separation was carried out on a Chiralpak AD column protected with a CN guard column [55].

Bonato and Paias developed two assay based on HPLC and capillary electrophoresis for the enantioselective analysis of OMZ in pharmaceutical formulations. (*R*)- and (*S*)-omeprazole were extracted from commercially available tablets using methanol:NaOH 2.5 M (90:10). Chiral HPLC separation was obtained on a Chiralpak AD column using hexane:ethanol (40:60) as the mobile phase and detection at 302 nm. The capillary electrophoresis procedure was carried out using 3% sulfated β-cyclodextrin in 20 mmol L^{-1} phosphate buffer, pH 4.0 and detection at 202 nm [56].

Cass et al. [57] report the analysis of plasma levels of omeprazole's enantiomers by direct sample injection. This was achieved by the use of a two dimensional chromatography system using a RAM (restricted access media) BSA C_8 column in the first dimension for extraction and clean-up and a polysaccharide column, under reversed-phase mode of elution, in the second dimension for the enantioseparation.

For the determination of OMZ in powder for injection and in pellets, Schubert et al. [58] proposed an analysis that were performed at room temperature on a reversed-phase C_{18} column of 250 nm × 4.6 mm i.d., 5 μm particle size.

The aim of other investigations is to study the stability of OMZ, in this sense, Mathew et al. studied the effect of pH on the stability and their quantified the drug in capsules [59] and Martins and Farinha used a system equipped with Nova-Pak C_{18} column using an eluent of phosphate buffer-acetonitrile and detection at 280 nm [60].

The objective of other assay is to use the microdialysis technique coupled to a validated microbore HPLC system to study the disposition and biliary excretion of OMZ after a single intravenous dose administration in rats. For this purpose, three microdialysis probes were simultaneously inserted into a rat for sampling biological fluids in the blood, brain and bile [61].

In Table 3 are shown the more relevant aspects of HPLC methods with UV detection for determination of OMZ.

4.2. Electrochemical detection

Persson and Wendsjo investigated the determination of OMZ in a reversed-phase LC system by electrochemical detection at a static mercury drop electrode. The mobile phase was composed of acetonitrile and phosphate buffer (pH 7.6). In the presence of oxygen dissolved in the mobile phase, OMZ could be indirectly detected at potentials between +0.2 and −0.8 V versus Ag/AgCl, where the compound is not electro-active [62].

On the other hand, HPLC employing coulometric detection is potentially more selective and sensitive than UV detection and may be applicable to determination of low levels of OMZ and corresponding impurities, degradates and metabolites. Sluggett et al. [63] report a novel method using coulometric detection in the oxidation mode. Coulometric detection was carried out at +800 mV using a porous carbon electrode.

4.3. Mixtures

Omeprazole and domperidone: A YMC-Pack C_{18} ODS-A, 10 μm, 120A column 250 mm × 4.6 mm i.d. in isocratic mode, with mobile phase 50 mM KH_2PO_4:acetonitrile (62:38) pH adjusted to 4.5 with 5% *o*-phosphoric acid were used. The flow rate was 1.0 mL min^{-1} and effluent was monitored at 220 nm. The retention time of domperidone and OMZ were 4.44 and 7.41 min, respectively [64].

Omeprazole and metronidazole: In order to determine the effect of OMZ on the gastric secretion of metronidazole in humans, sensitive and specific assays are needed to measure these drugs in plasma and gastric fluid. Yeung et al. [65] developed a simple HPLC assay for this purpose. The HPLC system consisted of a multi-phase column combining anion exchange and reversed phase separation (OmniPac Pax-500, Dionex), and a variable wavelength UV detector set at 254 nm. The mobile phase was a mixture of 0.1 M sodium phosphate buffer: methanol:acetonitrile (60:20:20) with final pH adjusted to approximately 7.0. The two drugs were extracted by adsorption

Table 3
High-performance liquid chromatography methods

Procedure	Analytical data	Samples	Reference
UV detection ($\lambda = 302$ nm)			
Mobile phase methanol–water (60:40) containing 1% triethylamine, adjusted to pH 7 with phosphoric acid (85%); flow rate 3 mL min^{-1} at a back pressure of approximately 340 kPa	Retention times: OMZ sulphone 3.1 min, OMZ 3.6 min, internal standard 4.9 min and OMZ sulphide 7.7 min; DL for omeprazole, sulphone and sulphide: 5, 30, 50 ng mL^{-1}, respectively	Human plasma and urine	[23]
Mobile phase methanol–acetonitrile–0.025 M phosphate buffer (40:8:52), pH adjusted to 7.40 with 85% phosphoric acid; flow-rate 1.1 mL min^{-1} with a back-pressure of l0–14 MPa and an operating temperature of 25 °C	Retention times: OMZ sulphone 7.5 min, OMZ 8.8 min, internal standard 13.0 min and OMZ sulphide 19.7 min; DL for omeprazole, sulphone and sulphide: at least 5, 10 and 7.5 ng mL^{-1}, respectively	Human plasma	[25]
Chromatographic separation for the plasma method (OMZ and sulphone) on a silica column with a mobile phase of methylene chloride containing 3.5% of a solution of 5% of concentrated ammonium hydroxide in methanol; for sulphide, methanol content decreased to 2.0%; flow-rate 1.5 mL min^{-1}; for 5OH-OMZ in plasma and OMZ and metabolites in urine, a reversed-phase system is used with a mobile phase containing acetonitrile and phosphate buffer pH 7.5 (30:70); flow-rate 1 mL min^{-1}	The minimum determinable concentration, defined as the level at which the relative standard deviation is l0–15%, is about 20 nmol L^{-1} for OMZ and sulphone, and about 50 nmol L^{-1} for sulphide; linearity ranges from 25–50 nmol L^{-1} to 50–100 pmol L^{-1} of plasma or urine	Plasma and urine	[26]
Separation column (stainless steel, 150 mm × 4.5 mm) packed with Polygosil C_{18}, 5 μm particles, protected by a guard column (stainless steel, 30 mm × 4.6 mm) packed with Spheri-5, RP18; mobile phase acetonitrile and phosphate buffer, pH 7.7; isocratic elution with 34% acetonitrile used for OMZ and sulphone in plasma and gradient elution system for three compounds in plasma and urine; gradient profile consisted of two isocratic parts and two linear steps within a period of 20 min. After 3 min with 25% acetonitrile, concentration was linearly increased to 40% during 1 min and kept there for 6 min; following 5 min acetonitrile content decreased to 25%, the system was equilibrated for 5 min before the next injection; in both systems the compounds were eluted within 10 min using a flow-rate of 1.5 mL min^{-1}	In plasma the limit of determination, defined as the concentration where the standard deviation is l0–15%, is 50 nmol L^{-1} for OMZ, 5OH-OMZ and sulphone; in the case of the urine method, where only 200 μL of sample is used, concentrations down to 200 nmol L^{-1} can be determined; linearity ranges from 0.05 to 50 μmol L^{-1} of plasma and 0.2–200 μmol L^{-1} of urine	Plasma and urine	[27]
Mobile phase acetonitrile–0.05 M phosphate buffer (pH 8.5) (25:75) at a flow-rate of 0.8 mL min^{-1}	Minimum determinable concentration was 10 ng mL^{-1} for all analytes in plasma and 5OH-OMZ in urine	Plasma and urine	[30]
Mobile phase 47% methanol and 53% of 0.1 M dipotassium hydrogenphosphate, pH 7.8; flow-rate 1.2 mL min^{-1} at 37 °C	Limit of quantitation 9.7 ng mL^{-1} and the calibration curve linear up to 1240 ng mL^{-1}	Human plasma	[31]
Mobile phase acetonitrile–phosphate buffer (24:76, pH 8), containing nonylamine at 0.015%; retention times 9.5 min for OMZ, 3.25 min for hydroxy, 7.4 min for sulphone and 6.27 min for internal standard (phenacetine)	Lowest limits for quantification were 60 ng mL^{-1} for every analyte; highest limit of detection was 960 ng mL^{-1}	Human plasma	[35]
Mobile phase phosphate buffer–acetonitrile (92:8, pH 7.0) for clean-up and phosphate buffer–acetonitrile–methanol (65:30:5, pH 6.5) for separation; total time for chromatographic separation 25 min, approximately	Linear range from 3 to 2000 ng mL^{-1} for OMZ, 3–500 ng mL^{-1} for 5OH-OMZ and 3–1000 ng mL^{-1} for OMZ sulphone; limits of quantification 3 ng mL^{-1} for OMZ and its metabolites	Human plasma	[36]
Using gradient elution; mobile phase A 22.0 mM phosphate mono basic, adjusted to a pH of 6.0 with diluted NaOH; this solution was filtered through a 0.45 μm membrane filter then mixed as 900 mL buffer to 100 mL methanol; mobile phase B 100 mL of the phosphate buffer as mobile phase A, mixed with 800 mL of acetonitrile, 100 mL of methanol, and 100 μL TFA; using a Zorbax® C_{18} (150 mm × 3.0 mm, 3.5 μm particle size) analytical column, with a Zorbax® C_{18} (12.5 mm × 4.6 mm) guard column	Linear range from 2 to 2000 ng mL^{-1} for all analytes; retention times 5OH-OMZ 4.3 min, Phenacetin (IS) 5.4 min; OMZ sulphone 7.4 min, OMZ 7.9 min, OMZ sulphide 10.4 min; low limit of quantification for all analyte compounds was 2 ng mL^{-1}, and upper limit of quantification was 2000 ng mL^{-1}	Human plasma	[37]

Table 3 (*continued*)

Procedure	Analytical data	Samples	Reference
Hypersil ODS2 C_{18} column (250 mm × 4.6 mm, 5 μm) fitted with a Phenomenex guard column packed with octadecyl C_{18}; mobile phase 50 mM potassium dihydrogen phosphate buffer (pH 7.1, contained 0.7% TEA) and acetonitrile (75:25); at a flow rate of 1.0 mL min^{-1} at 25 °C	Linear range from 0.02 to 3 μg mL^{-1}	Rat plasma	[38]
Eluent (50 μL) injected on a μBondapak C_{18} reversed-phase column (4.6 mm × 250 mm; 10 μm); mobile phase 0.05 M phosphate buffer (pH 7.5) and acetonitrile (75:25) at a flow rate of 0.8 mL min^{-1}	Linear range from 50 to 2000 ng mL^{-1}; minimum detection limit OMZ and OMZ sulphone 10 ng mL^{-1} and 5OH-OMZ 15 ng mL^{-1}	Human plasma	[39]
Mobile phase sodium phosphate mono-basic (pH 7.2; 20 mM)–acetonitrile (70:30); mobile phase degassed prior to use under vacuum by filtration through a 0.2 mm Millipore membrane and during the chromatographic process with helium; flow-rate 0.5 mL min^{-1}	Linear range between 5 and 500 ng mL^{-1}; limit of quantitation 5 ng mL^{-1}	Human plasma	[40]
Mobile phase 23% acetonitrile and 77% of 30.4 mM Na_2HPO_4 and 1.76 mM KH_2PO_4 solution, pH 8.4, in which a gradient elution was used to linearly change solvent composition to 33% acetonitrile and 67% phosphate buffer during the first minute; at 294 nm for the IS; total analysis time 4 min	Lower limit of quantitation 10 ng mL^{-1} and the calibration function is linear to 2000 ng mL^{-1}	Human plasma	[41]
Using ethanol:hexane (70:30) as the mobile phase	Linear range 10–1000 ng mL^{-1} for each enantiomer; quantitation limit 5 ng mL^{-1}	Human plasma	[55]
Using acetonitrile–water (60:40) as eluent	Linear range 0.05–4.80 μg mL^{-1}; DL 0.0063 μg mL^{-1} for each enantiomer	Human plasma	[57]
Mobile phase methanol–water (90:10) was pumped at a constant flow rate of 1.5 mL min^{-1}	Linear range 32–48 μg mL^{-1}	Bulk and injectable preparations	[58]

onto a C_2-bonded silica gel solid phase extraction column, and eluted with methanol.

Omeprazole, lansoprazole and pantoprazole: Ekpe and Jacobsen developed an HPLC method capable of simultaneous quantitation of three compounds; also determined the effect of various salts and pH on the stability of the three proton pump inhibitors. The three compounds were monitored at 280 nm using Zorbax Eclipse XDB C_8 (5 μm, 150 cm × 4.6 mm i.d.) and a mobile phase consisting of 70:30 phosphate buffer:acetonitrile with the pH adjusted to 7.0 with phosphoric acid [66].

More recently, El-Sherif et al. [67] proposed a method for the quantitative determination of the three compounds in the presence of their acid-induced degradation products. The three compounds were monitored at 280 nm using Nova-Pak C_{18} column and a mobile phase consisting of 0.05 M potassium dihydrogen phosphate:methanol:acetonitrile (5:3:3).

Omeprazole, caffeine, flurbiprofen, dextromethorphan and midazolam: An efficient, fast and reliable analytical method was developed for the simultaneous evaluation of the activities of five major human drug metabolising cytochrome P450 (1A2, 2C9, 2C19, 2D6 and 3A4) with a cocktail approach including five probe substances, namely caffeine, flurbiprofen, omeprazole, dextromethorphan and midazolam [68]. All substances were administered simultaneously and a single plasma sample was obtained 2 h after the administration. Plasma samples were handled by liquid–liquid extraction and analysed by gradient HPLC coupled to UV and fluorescence detectors. The chromatographic separation was achieved using a Discovery semi-micro HS C18 HPLC column (5 μm particle size, 150 mm × 2.1 mm i.d.) protected by a guard column (5 μm particle size, 20 mm × 2.1 mm i.d.) The mobile phase was constituted of a methanol, acetonitrile and 20 mM ammonium acetate (pH 4.5) with 0.1% triethylamine mixture and was delivered at a flow rate of 0.3 mL min^{-1}. All substances were separated simultaneously in a single run lasting less than 22 min.

5. Liquid chromatography–mass spectrometry

While several methods for the determination OMZ and OMZ-OH have been described in the literature [26,27,29], these assays suffer from inadequate sensitivity and the use of a complex automated sample preparation system [27], long analysis times [29], or the use of toxic halogenated solvents during sample preparation [26,29]. A simultaneous assay for the determination of OMZ and OMZ-OH in human plasma using SPE for analyte isolation using a polymeric sorbent based cartridge and HPLC with tandem mass spectrometry (MS–MS) is described by Woolf and Matuszewski [69]. The HPLC mobile phase consisted of a mixture of acetonitrile:water (21:79) containing 10 mM ammonium hydroxide. The apparent pH of the mobile phase was adjusted to 8.5 with formic acid prior to use. A Sciex API III+ tandem mass spectrometer equipped with a heated nebuliser atmospheric pressure chemical ionization interface was used as a detector and was operated in the positive ion mode. The assay was used to determine the cytochrome P450 2C19 phenotype of subjects participating in clinical trials of compounds

under development. The method was validated in the concentration range of 10–500 ng mL^{-1} plasma with adequate assay precision and accuracy, but the sensitivity was insufficient for pharmacokinetic studies and the run time of 11 min was rather long.

For resolving these problems, Wang et al. [70] proposed a rapid, sensitive and selective liquid chromatography–electrospray mass spectrometry (LC–ESI-MS) method for the quantitation of OMZ. The method was applied to a bioequivalence study of two oral formulations of OMZ. The analyte and internal standard sildenafil are extracted from plasma by liquid–liquid extraction using diethyl ether:dichloromethane (60:40) and separated by reversed phase HPLC using acetonitrile:methanol:10 mM ammonium acetate (37.5:37.5:25) as mobile phase. Detection is carried out by multiple reactions monitoring on a Q TRAP™ LC/MS/MS system. The method has a chromatographic run time of 3.5 min and is linear within the range 0.50–800 ng mL^{-1}.

A method has been developed and validated by Frerichs et al. [71] for the quantitation of midazolam, alphahydroxymidazolam, omeprazole, and hydroxyomeprazole from one 250 μL sample of human plasma using high performance liquid chromatography coupled to tandem mass spectrometry. The method was validated for a daily working range of 0.400–100 ng mL^{-1}, with limits of detection between 2 and 15 pg mL^{-1}. The inter-assay variation was less than 15% for all analytes at four control concentrations and the samples were stable for three freeze-thaw cycles under the analysis conditions and 24 h in the post-preparative analysis matrix. This method was used to analyse samples in support of clinical studies probing the activity of the cytochrome P450 enzyme system.

An enantioselective assay of OMZ in blood plasma using normal-phase LC on a Chiralpak AD column and detection by MS is described by Stenhoff et al. [72]. OMZ is extracted by a mixture of dichloromethane and hexane and, after evaporation, redissolution and injection, separated into its enantiomers on the chiral stationary phase. Detection is made by a triple quadrupole mass spectrometer, using deuterated analogues as internal standards. The method enables determination in plasma down to 10 nmol L^{-1} and shows excellent consistency suited for pharmacokinetic studies in man.

Because the metabolism of OMZ is mainly catalyzed by cytochrome P450 (CYP) 3A4 and CYP2C19, the genetic polymorphism of CYP2C19 could be of clinical concern in the treatment of acid-related diseases with OMZ. Therefore, a reliable method for OMZ phenotyping is desirable in clinical situations. The study, carried out by Kanazawa et al. [73], demonstrates the analysis of OMZ in human plasma as a probe drug of CYP2C19 phenotyping by liquid chromatography–three-dimensional quadrupole mass spectrometry (LC–3DQMS) with a sonic spray ionization (SSI) interface. The analytical column was YMC-Pack Pro C_{18} (50 mm × 2.0 mm i.d.) using acetonitrile:50 mM ammonium acetate (pH 7.25) (1:4) at a flow-rate of 0.2 mL min^{-1}.

Only a few methods exist which are capable of quantitating all three analytes (OMZ and its two major metabolites) in plasma. Most HPLC methods used are applied to phenotyping, and are not sensitive enough for pharmacokinetic studies. Even if low LOQs are specified in the method description, validation data are shown for much higher concentrations. One tandem mass spectrometric method described already is also too insensitive [72]. LC–MS–MS has been used for the determination of omeprazole and 5-hydroxyomeprazole [68,70] with high sensitivity (10 or 0.8 ng mL^{-1}, respectively), but the sulphone was not determined with this method.

In order to fill this methodological gap, Hofmann et al. [74] developed a sensitive and selective LC–MS method for the simultaneous determination of omeprazole, 5-hydroxyomeprazole and omeprazole-sulphone in human plasma. The method was applied to a pharmacokinetic study with esomeprazole in patients with gastroesophageal reflux disease (GERD). Following liquid–liquid extraction HPLC separation was achieved on a ProntoSil AQ, C_{18} column using a gradient with 10 mM ammonium acetate in water (pH 7.25) and acetonitrile. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH^+ ions, *m/z* 346 for omeprazole, *m/z* 362 for 5-hydroxy-omeprazole and omeprazol-sulphone and *m/z* 300 for the internal standard (2-{[(3,5-dimethylpyridine-2-yl)methyl]thio}-1*H*-benzimidazole-5-yl) methanol. The limit of quantification (LOQ) achieved with this method was 5 ng mL^{-1} for 5-hydroxyomeprazole and 10 ng mL^{-1} for omeprazole and omeprazole-sulphone using 0.25 mL of plasma.

Recently, the use of multiple probe drugs, i.e. a 'cocktail' approach, has become popular in pharmacogenetic studies as this provides a high-throughput approach in evaluating CYP isozyme activities. Recently, Yin et al. [75] developed a five-drug cocktail, consisting of caffeine, tolbutamide, omeprazole, debrisoquine and midazolam, for phenotyping of CYP1A2, 2C9, 2C19, 2D6 and CYP3A isozyme activity, respectively, and LC–MS method, for rapid determination of five cytochrome P450 (CYP) probe drugs and their relevant metabolites in human plasma and urine, is described. The five specific probe substrates/metabolites together with the internal standards (phenacetin and paracetamol), in plasma and urine, were extracted using solid-phase extraction. The chromatography was performed using a C_{18} column with an isocratic mobile phase consisting of acetonitrile and 0.1% formic acid in water (70:30). The triple-quadrupole mass spectrometer was operated in both positive and negative modes, and multiple reaction monitoring was used for quantification.

Chung et al. [76] describes a convenient method for the extraction and detection of eight anti-ulcer drugs simultaneously in horse urine, a relatively complex and viscous matrix, using a single-step liquid-liquid extraction followed by LC–MS. Anti-ulcer drugs was isolated from horse urine by salting out and liquid–liquid extraction. Detection of these drugs at concentrations below 1 ng mL^{-1} could be achieved using LC–MS–MS in the positive atmospheric pressure chemical ionization (APCI) mode.

Bioanalytical methods using liquid–liquid extraction (LLE) and liquid chromatography with electrospray tandem mass spectrometry are widely used. The organic extracts need to be evaporated and reconstituted, hampering further improvement of throughput and automation. In a study, Song and Naidong

demonstrated a novel approach of eliminating these two steps in 96-well LLE by using hydrophilic interaction chromatography with MS–MS on silica column with high organic/low aqueous mobile phase. OMZ, its metabolite 5-OH omeprazole, and internal standard desoxyomeprazole, were extracted from 0.05 mL of human plasma using 0.5 mL of ethyl acetate in a 96-well plate. A portion (0.1 mL) of the ethyl acetate extract was diluted with 0.4 mL of acetonitrile and 10 μL was injected onto a Betasil silica column (50 mm × 3.0 mm, 5 μm) and detected by API 3000 and 4000 with (+) ESI. Mobile phase with linear gradient elution consists of acetonitrile, water, and formic acid (from 95:5:0.1 to 73.5:26.5:0.1 in 2 min). The flow rate was 1.5 mL min^{-1} with total run time of 2.75 min. The method was validated for a low limit of quantitation at 2.5 ng mL^{-1} for both analytes [77].

Also, recently Xu et al. [78] develop an LC–MS method for the simultaneous determination of midazolam, dextromethorphan and omeprazole in rat plasma. After addition of 10 μL of diazepam solution (5 μg mL^{-1}, internal standard) to 0.1 mL plasma and alkalization with 100 μL of 0.2 mol L^{-1} Na_2CO_3, plasma was extracted with 5 mL of ether. Three milliliters of organic layer was then transferred and evaporated to dryness. The residue was reconstituted in 200 μL methanol and 5 μL of aliquots was injected into an ODS C_{18} (250 mm × 2.0 mm, 5.0 μm) column. The mobile phase consisted of 0.01% NH_4Ac–methanol (30:70) at a flow rate of 0.2 mL min^{-1}. The elution from the HPLC column was plumbed directly into ESI probe. Analysis in the mass spectrometer was operated in the selected ion monitoring mode. The mass spectrometer was operated in SIM *m/z*: 326.0 for midazolam, *m/z*: 272.1 for dextromethorphan, *m/z*: 346.0 for omeprazole, and *m/z*: 284.9 for diazepam. The established LC–MS is suitable for pharmacokinetic study of midazolam, dextromethorphan and omeprazole and could be applied in high through-put screen of new drugs as "cocktail" research.

6. Supercritical fluid chromatography

del Nozal et al. [79] describe the enantiomeric separation of OMZ and several benzimidazoles on the amylose based column Chiralpak AD studying the effect of the organic modifier and the temperature on the retention and enantio-resolution. The best results are allowed with alcohol type modifiers.

7. Thin layer chromatography (TLC)

Few methods use this technique for determination OMZ [80–82]. OMZ is separated from other constituents on activated pre-coated silica gel (60F254) plates using methanol:water (2:1) as mobile phase. The spots were measured in situ using spectrodensitometer at 302 nm [80] and a method has been developed for separation of omeprazole and pantoprazole, and their impurities omeprazole-sulphone and *N*-methylpantoprazole, by HPTLC [82]. The mobile phase chloroform-2-propanol–25% ammonia-acetonitrile enables good resolution of large excesses of the drugs from the possible impurities.

8. Capillary electrophoresis

HPLC using chiral stationary phases was the preferred technique for the enantioselective analysis, until the advent of capillary electrophoresis (CE) in the last decade. The main advantages of CE for the enantioselective analysis of chiral drugs are the extremely high efficiency, instrumentation simplicity, low sample and reagent consumption and speed in method development and analysis. In addition, CE is a complementary technique to HPLC particularly for the analysis of charged and polar compounds.

As described above, several papers have been dedicated to the OMZ enantiomers separation in biological fluids using HPLC techniques. Recently, CE has been widely used for enantiomer separation for a large number of compounds including many of pharmaceutical interest. Rapid chiral analyses with high efficiency and low cost were achieved by CE by simply adding the chiral selector to the background electrolyte or binding it to the capillary wall or to a stationary phase. The chiral selectors used for this purpose include cyclodextrins (CDs) and derivatives, chiral crown ethers, noncyclic oligosaccharides and polysaccharides, macrocyclic antibiotics, proteins and peptides, metal complexes, and chiral surfactants.

Some works have described different enantioselective separation phases by EC for the enantiomers separation of a variety of basic drugs, that include omeprazole [83], comparing the HPLC techniques with regard to CEC [84]. Eberle et al. [85] studied the possibilities of the separation of the pantoprazole enantiomers and related sulphoxides (OMZ) by capillary zone electrophoresis (CZE) using bovine serum albumin (BSA) as chiral selector. Enantiomeric discrimination was observed only in a narrow pH range of 7–8. In order to improve the peak shape of the analytes on the resolution, 1-propanol (7%) was added to the electrolyte solution (10 mM phosphate, pH 7.4). The optimized method was validated for pantoprazole and the authors concluded that BSA concentration had a dramatic influence on the separation.

Cyclodextrins, a family of torus-shaped maltooligosaccharides, have found many applications in recent years because of their ability to form inclusion complexes with a large number of molecules, ranging from small linear to large polyaromatic. Although their main applications are in the pharmaceutical, cosmetic, food, and agrochemical industries, CDs have also proved of great utility in analytical chemistry, especially in providing separations of positional and optical isomers. Berzas Nevado et al. [86] developed a simple and rapid CZE method for the separation of OMZ enantiomers using CDs as chiral selector. The proposed method was optimized for experimental parameters such as type and concentration of the β-cyclodextrin (β-CD) used, buffer concentration, and capillary temperature. Analysis times, shorter than 8 min were found using a background electrolyte solution consisting of 40 mM phosphate buffer adjusted to pH 2.2, 30 mM β-cyclodextrin and 5 mM sodium disulphide, hydrodynamic injection, and 15 kV separation voltages. Detection limits were evaluated on the basis of baseline noise and were established 0.31 mg L^{-1} for the omeprazole enantiomers.

Other study demonstrates the development and validation of a non-aqueous capillary electrophoresis (NACE) method for enantiomeric determination of OMZ and its metabolite 5-hydroxyomeprazole [87]. Heptakis-(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin (HDMS-β-CD) was chosen as the chiral selector in an ammonium acetate buffer acidified with formic acid in methanol. Parameters such as CD concentration, concentration of buffer electrolyte, voltage and temperature were studied in order to optimize both the enantioresolution and migration times. An experimental design was utilized for method optimization, using software Modde 5.0. The limits of detection for the four enantiomers were in the range from 45 to 51 μM and the limits of quantification were between 149 and 170 μM with UV detection at 301 nm.

Capillary zone electrophoresis (CZE) has been proposed for the determination of OMZ in human plasma using a running buffer composed of 50 mM phosphate–12.5 mM borate at pH 10.1 [88] and for the simultaneous analysis of OMZ and lansoprazole in capsules [89], a phosphate buffer (50 mM; pH 9) was used as electrophoretic electrolyte.

Finally Pérez-Ruiz et al. [90] describe a sensitive new method. It involves an automated SPE procedure and capillary electrophoresis with UV detection. OMZ, hydroxyomeprazole and omeprazole-sulphone could be separated by micellar electrokinetic capillary chromatography using a background electrolyte composed of 20 mM borate buffer and 30 mM sodium dodecyl sulfate, pH 9.5. The isolation of OMZ and its metabolites from plasma was automatically accomplished with an original SPE procedure using surface-modified styrene–divinylbenzene polymer cartridges.

9. New trends in the determination of omeprazole

Several attempts have been made to understand the chemical conversions and the mechanism of action of OMZ. Among others, these have included: isolation, structure elucidation, and characterization of both intermediates and decomposition products in the acidic media. As can be seen above, reversed phase HPLC coupled with UV detection, and indirect UV spectrophotometry have been used to understand the degradation of OMZ in acidic media; a recent work [17] utilized DPP, at the static mercury drop electrode, for monitoring simultaneously the current(s) of omeprazole decay with time, and the appearance of all degradation products in the absence of thiol. The fast, sensitive, and specific technique facilitated an accurate determination of a spectrum of compounds related to omeprazole decomposition products.

Methods that simultaneously measure omeprazole and 5-hydroxyomeprazole and omeprazole-sulphone have been published [33,55]. Rezk et al. [37] summarize the development and validation of the simultaneous determination of omeprazole and its three metabolites in small sample volumes after a simple and highly reproducible liquid–liquid extraction procedure. The assay has been validated with respect to accuracy, precision, linearity and limit of detection, recovery and stability. It has been successfully applied to clinical samples from healthy volunteer subjects. This method has many clinical applications, as it is simple, highly sensitive, and inexpensive.

On the other hand, for separating and quantitating the proton-pump inhibitors lansoprazole, omeprazole and pantoprazole in pharmaceutical preparations (tablets and capsules), El-Sherif et al. [67] proposed reversed-phase, isocratic HPLC method on Waters Nova-Pak C_{18} column, that have been developed and validated, the concomitant quantitation provides significant decrease in sample preparation, instrument run time, solvent and drug waste over the separation methods of analysis. Moreover, the method could separate the intact drugs in presence of more than seven main degradation products; indicating system suitability and efficient separation.

During the last decade, quantification of low molecular weight molecules using liquid chromatography–tandem mass spectrometry in biological fluids has become a common procedure in many preclinical and clinical laboratories. The combination of liquid chromatography and mass spectrometry (LC–MS) has been used for omeprazole and metabolites [69,70,73,74,78], for example, a very important method has been developed for the simultaneous determination of OMZ and its two major metabolites 5OH-OMZ and OMZ sulphone in human plasma using liquid–liquid extraction and HPLC–electrospray mass spectrometry [74]. Sensitivity, selectivity and reproducibility allow for the application in pharmacokinetic studies. In 10 patients with GERD the most relevant pharmacokinetic parameters of the three compounds have been evaluated following single and multiple therapeutic dosing.

On the other hand, esomeprazole is the first proton pump inhibitor developed as an optical isomer (*S*-omeprazole) for the treatment of acid-related diseases. Esomeprazole is a potent inhibitor of gastric acid secretion and accumulates in the acidic compartment of the parietal cells where the molecule is transformed to its active sulphenamide form. Esomeprazole is metabolized to two major metabolites, 5-hydroxyesomeprazole and esomeprazole sulphone. Esomeprazole does not undergo chiral inversion in vivo and therefore esomeprazole can be determined using the same methodology as for its racemate, omeprazole. Enantioselective methods for omeprazole by liquid chromatography have also been presented which employ mass spectrometric [72] or UV-detection [57]. Reported methods for omeprazole and its two major metabolites require a sample volume of 0.2–1.0 mL and a chromatographic run time of 16–60 min. Hultman et al. [91] present a method based on LC–MS–MS after liquid–liquid extraction of esomeprazole and the two major metabolites using a plasma volume of 25 μL from human, rat or dog and a total liquid-chromatographic run time of about 6 min. The small sample volume has made the method adequate for toxicokinetic evaluation in rat and dog puppies and for pharmacokinetic evaluation in children (pre-term and neonates).

Also, capillary electrophoresis (CE) has gained significant acceptance in the analytical laboratory owing to its many advantageous features, such as extremely high efficiency, high resolution, rapid analysis and small consumption of sample and reagents. An NACE method has been developed and validated for the separation of an omeprazole racemate and also the racemate of its metabolite 5-hydroxyomeprazole [87]. Baseline

separation and detection of the two racemates was achieved in a single run. Problems with current break down in NACE have been diminished by lowering the temperature on both the tray and the capillary, decreasing the effect of Joule heating within the capillary. Another advantage of this was the improved resolution. When using UV detection, the value for the detection limit is high, therefore MS is currently being investigated as an alternative detector.

Micellar electrokinetic capillary chromatography (MEKC), one of the most important modifications of CE, has proved to be an excellent alternative to HPLC for drug analysis. MEKC is faster and less costly than HPLC and for some analyses, it has replaced HPLC as the method of choice. Pérez-Ruiz et al. [90] develop a rapid and straightforward CE method for the direct determination of OMZ and its metabolites. The method also involves an automated and efficient SPE procedure for the pretreatment of the plasma samples and MEKC analysis. The assay was validated by determining its accuracy, precision, linearity and selectivity. The 6.5 min run time of the assay is much shorter that described for HPLC assays, thus allowing increased sample throughput. Additionally, the SPE step of the assay is fully automated, permitting the SPE and CE systems to work concurrently, i.e. while plasma sample-1 was being analysed, plasma sample-2 was simultaneously purified from the biological matrix by the SPE system. The method has proven to be a suitable alternative for the analysis of plasma samples collected during human clinical studies with OMZ.

10. Conclusions

Most of the reported methods are HPLC which require elaborate procedures. The electrochemical methods are generally less sensitive. The conventional UV methods suffer from interference due to UV absorbing compounds in the determination of the cited drug. The few reported visible spectrophotometric methods are mainly concerned with charge transfer complexation with different electron acceptors, which give similar reaction with all basic compounds or concerned with the reducing activity of OMZ. In the last years, derivative techniques in UV spectrophotometry have been used as separative methods for the analysis of drug mixtures, determination of degradation products as well as in stability studies.

HPLC and CE methods were well suited for the enantioselective analysis of OMZ in pharmaceutical formulations. Chiral CE is more versatile and less expensive than HPLC using chiral columns, since several expensive columns are required to cover a reasonably wide application range and column lifetime tends to be relatively short. In addition, chiral HPLC requires a large volume of organic solvents. On the other hand, the HPLC method is more sensitive and resulted in a better resolution of OMZ enantiomers.

The conclusion is that the ions-pray LC–MS–MS method has advantages in providing shorter analytical run time, higher selectivity and much lower limit of quantification compared with previous analytical methods.

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