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Improving CYP2C19 phenotyping using stereoselective omeprazole and 5-hydroxy-omeprazole metabolic ratios

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Abstract

Omeprazole (OME) is a CYP2C19 phenotyping probe, marketed as a racemic *(S)/(R)* mixture or as an S-enantiomer. Both CYP2C19 and CYP3A4 enzymes mediate (R)-OME hydroxylation to (R)-5-hydroxyomeprazole, while (S)-OME is exclusively hydroxylated via CYP2C19. This study investigates OME and its 5-hydroxymetabolite enantiomers' pharmacokinetics using data from two studies involving healthy volunteers. In Study A, volunteers received OME alone in Session 1, OME combined with voriconazole and fluvoxamine in Session 2 and finally OME with rifampicin in Session 3. In Study B, volunteers received OME alone in Session 1, OME combined with voriconazole in Session 2 and finally OME with fluvoxamine in Session 3. Despite low metabolic ratio values of (S)-OME, detectable modulation of CYP2C19 activity suggests both (R)- and (S)-OME isomers could effectively assess CYP2C19 activity. Further research is needed for precise cut-offs in different phenotype groups.

KEYWORDS
5-hydroxy-omeprazole, CYP2C19, enantiomers, omeprazole, phenotyping

Plain English Summary

Omeprazole is widely used for CYP2C19 phenotyping. When administered as a racemic mixture, (R-S)-omeprazole undergoes stereoselective metabolism and is primarily metabolized to (R)-5-hydroxyomeprazole via CYP2C19/CYP3A and (S)-5-hydroxyomeprazole via CYP2C19. The goal of our study was to provide additional data concerning omeprazole stereoselective hydroxylation and evaluate the potential use of omeprazole enantiomeric metabolic ratio (R or S) to improve CYP2C19 phenotyping. We demonstrated that both omeprazole enantiomers could be used for CYP2C19 activity assessment. However, the (S)-enantiomer metabolic ratio at 2 h after omeprazole administration and the AUC ratio performed better, particularly for induction. We also proposed validated cut-offs for CYP2C19 phenotyping using (S)-omeprazole.

1 | INTRODUCTION

Cytochrome P450 2C19 (CYP2C19) enzyme is estimated to metabolize approximately 15% of all marketed drugs, among which proton pump inhibitors, antiplatelet agents, antidepressants, beta-blockers, benzodiazepines and many other compounds.[1,2]

CYP2C19 metabolism is largely affected by genetic polymorphisms, leading to serious therapeutic complications.[3] Moreover, drug–drug interactions (DDIs) are recognized as a major cause in CYP2C19 activity variability. The Dutch Pharmacogenetic Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) have established guidelines for several CYP2C19 substrates based on genetic test results.[4,5] However, while genotyping provides valuable information about genetic predisposition, it does not capture the dynamic aspects of enzyme activity influenced by endogenous and environmental factors. The standard approach for measuring CYP2C19 activity is phenotyping. In addition to the information provided by genotyping, phenotyping provides clinically relevant information about the in vivo activity of enzymes at a given moment and considers the combination of genetic, endogenous and environmental factors. CYP450 phenotyping consists of administering a probe drug metabolized by a specific cytochrome and assessing different pharmacokinetic parameters of the probe drug and the related metabolite, allowing the definition of an individual metabolic profile.[6–8]

One of the first strategies developed to assess CYP2C19 activity was using mephenytoin as a probe drug. However, mephenytoin's narrow therapeutic index and association with a higher risk of adverse effects, mainly severe toxicity in CYP2C19 poor metabolizers (PMs), made it less ideal for routine phenotyping. Over time, there has been a transition from utilizing mephenytoin to omeprazole (OME) as a probe drug for CYP2C19 phenotyping.[9] Unlike mephenytoin, OME has a wider therapeutic window and is better tolerated, making it a safer and more practical alternative for CYP2C19 phenotyping.

OME carries a chiral centre (the sulfinyl group) and was initially marketed as a racemic mixture of *(R)-(+)-* and *(S)-(−)*-enantiomers before studies revealed stereoselective enzyme-catalysed metabolism.[10] When administered as a racemic mixture, OME undergoes stereoselective metabolism and is primarily metabolized to 5-hydroxyomeprazole (referred to in this article as OH-OME), 3-hydroxyomeprazole, 5-O-Desmethylomeprazole and OME sulfone (OME-SUL) by CYP2C19 and CYP3A4 (Figure 1). *(R)*-OME hydroxylation to *(R)*-5-hydroxyomeprazole is mediated by both CYP2C19 and CYP3A4, in contrast to *(S)*-OME, which is exclusively hydroxylated to 5-hydroxyomeprazole via CYP2C19. This makes the *(S)*-isomer a promising candidate for CYP2C19 activity assessment. Another rationale for this work is that esomeprazole (=*(S)*-OME) is one of the most widely prescribed drugs. Using esomeprazole as a means of CYP2C19 phenotyping could prevent treatment interruption for some patients, as is currently the case. Although all these advantages are encouraging, it is important to note that *(S)*-isomer clearance is described to be significantly lower than that of the *(R)*-isomer.[11]

Our work aimed to provide additional data concerning OME stereoselective hydroxylation to the OH-OME enzyme-catalysed metabolism pathway via CYP2C19. The study analysed samples from healthy volunteers receiving oral OME racemate alone, after CYP2C19 enzyme inhibition using either voriconazole and fluvoxamine simultaneously or separately to evaluate the importance of the CYP3A pathway in *(R)*-OME metabolism, and after CYP2C19 induction using rifampicin. The purpose was to assess inhibition and induction impact on *(R)*- and *(S)*-metabolic ratio, hence CYP2C19 activity and OME enantiomers' suitability for CYP2C19 phenotyping.

2 | MATERIALS AND METHODS

2.1 | Study design and population

The first set of data (Study A) was from a previous study (ClinicalTrials.gov Identifier NCT01731067) involving

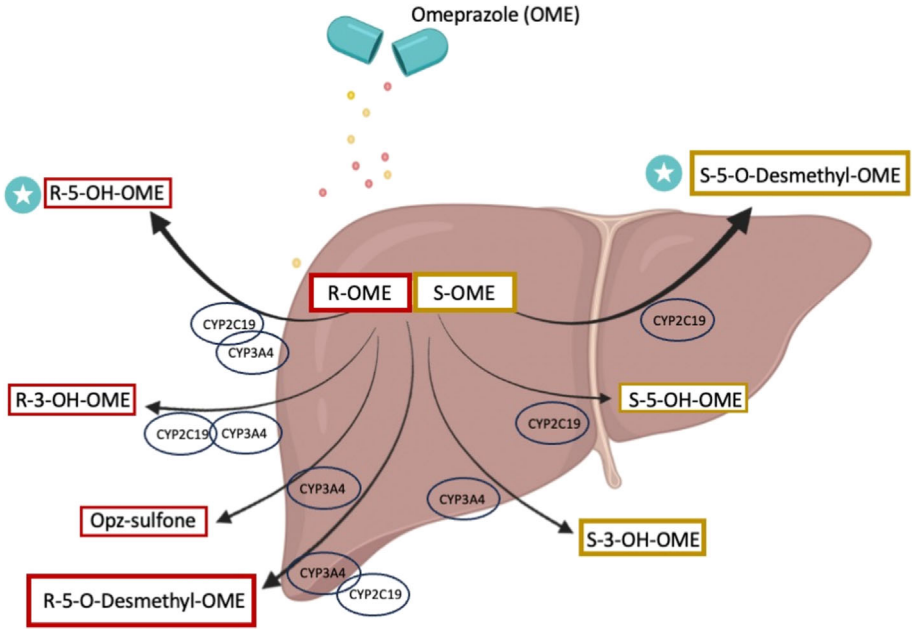

FIGURE 1 Schematic representation of the metabolic pathways of omeprazole. Blue star: major metabolism pathway.

10 healthy male volunteers who received sequentially 5 mg OME racemate for CYP2C19 phenotyping (Session 1: control session). During the inhibition session, participants received 5 mg of OME after taking fluvoxamine (50 mg) 12 h before OME administration and fluvoxamine (50 mg) and voriconazole (400 mg) 2 h before OME administration (Session 2: inhibition session).

Volunteers in Study A were genotyped for CYP2C19, and only normal or rapid metabolizers (*1/*1 and *1/*17) were included in the clinical trial.

Co-administration of voriconazole and fluvoxamine during the same session was designed to inhibit cytochromes 2C9, 2C19 and 3A4 (by voriconazole) and CYP1A2 (by fluvoxamine). OME 5 mg was also administered after pretreatment with rifampicin (600 mg, one tablet every evening for 7 days until the day before OME administration) (Session 3: induction session). Venous blood samples (3 ml) were taken before (Time 0) and 0.5, 1, 2, 3, 4, 6 and 8 h after OME administration and were used to extract the plasma. Corresponding dried plasma spots (DPS) were analysed in this study. Further details of the study have been described elsewhere.[6]

A second data set (Study B) was extracted from a recent phase 1, open-label, monocentric parallel study in healthy volunteers (ClinicalTrials.gov Identifier: NCT05264142). Included subjects were allocated in three groups according to their genotype. Only the study's normal metabolizer-genotyped volunteers ($n = 15$) were considered (CYP2C19*1/*1) in this work.

In the first session, Study B volunteers received 10 mg OME for CYP2C19 phenotyping (control session or Session 1). During the inhibition sessions, participants received 10 mg of OME after taking voriconazole (400 mg 2 h before OME administration) at Session 2 (voriconazole inhibition session) and fluvoxamine (50 mg, 12 and 2 h before OME administration) at Session 3 (fluvoxamine inhibition session). Dried blood spots (DBS) were taken before (Time 0) and 2, 3, 6 and 8 h after probe administration.

Given that voriconazole acts as both a moderate inhibitor of CYP2C19 and a strong inhibitor of CYP3A4, and given the involvement of CYP3A4 in CYP2C19-mediated hydroxylation of *(R)*-OME, a comparison was made with dataset B, where CYP2C19 inhibition was performed in two steps, using voriconazole and fluvoxamine separately. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[12]

2.2 | Analytical method

OME and OH-OME concentrations in plasma and blood were determined using an in-house validated stereoselective 2D high-performance liquid chromatography–mass spectrometry analysis.[13]

For sample preparation, 10 mm diameter discs covering the entire DPS (Study A) and 8 mm diameter discs covering the entire DBS (Study B) were cut and folded into the bottom of individual LC vials containing a 300 µL inert insert. For extraction, methanol (100 µL) containing the internal standard was added to each vial. The extracted solution was diluted twofold with water (100 µL) before injection.

DPS and DBS analysis was carried out using an LC–MS/MS system comprising a QTRAP 6500 mass

spectrometer from SCIEX (Toronto, Canada) and an Agilent 1290 Infinity II LC system with an additional Agilent 1100 LC pump (Agilent, Palo Alto, USA) for the first and second dimensions, respectively. Non-stereoselective online extraction and enrichment were performed using a Discovery HS C18 short reversed-phase column (20 × 2.1 mm ID, 5 µm, 120 Å, Supelco) equipped with an online filter (size 0.004-in. ID × 2 µm depth × 0.5 µm porosity, Phenomenex). Stereoselective separation was carried out on a chiral column based on chlorinated phenylcarbamate cellulose (150 × 2 mm ID, 3 µm, 1000 Å, Lux Cellulose-4, Phenomenex). The mobile phases for the first reversed-phase dimension consisted of (A1) water and (B1) methanol, and (A2) water and (B2) acetonitrile for the second stereoselective dimension. Both columns were operated at a flow rate of 300 µL/min. The method used was fully validated according to international criteria.[15]

2.3 | Statistical analysis

The single-point metabolic ratios (MRs) were determined as the concentration ratio between the metabolite and the administered substance: 5-hydroxyomeprazole/omeprazole (OH-OME/OME) at a given time-point. The pharmacokinetic parameters were estimated by standard non-compartmental methods using Pkanalix 2023R1 (Lixoft SAS, a Simulations Plus company). The area under the curve (AUC) ratios were determined as the ratio between the AUC of the metabolite and the AUC of the administered probe.

The results are presented as a median or mean ratio and a 90% confidence interval (CI). The MR and AUC ratios were compared using a nonparametric Wilcoxon *t*-test. The correlation between AUC ratios and single-point MRs of volunteers was compared using the Pearson test. A probability of $p < 0.05$ was considered statistically significant.

All statistical analyses were performed using the DATAtab Online Statistics Calculator (DATAtab Team [2023] DATAtab e.U. Graz, Austria).

3 | RESULTS

3.1 | Subjects

For Study A, 10 healthy male volunteers were enrolled and completed the study. They were of Caucasian ethnicity, aged between 20 and 36 years (median age 23) with a body mass index (BMI) between 19.9 and 24.4 (median BMI of 22.0) (details described in the research article by Bosilkovska et al.[6]).

For Study B, 15 healthy males and females were enrolled and completed the study. All participants were of Caucasian ethnicity, with eight females and seven males aged between 21 and 47 years (median age 28) with a BMI between 18.7 and 31.8 (median BMI 21.6).

3.2 | Study A

3.2.1 | Pharmacokinetic data

As shown in Figure 2A and Table 1, the pharmacokinetic profiles of *(S)*-OME and *(R)*-OME were similar in distribution and elimination. No statistically significant differences were observed between either enantiomer with respect to time to maximum concentration (T_{max}: 2 h) at each session. Maximum concentration (C_{max}) and AUC were significantly different for OME enantiomers between the control session as well as for the inhibited and induced one (C_{max} of 87.11, 415.34 and 8 vs. 126.52, 446.1 and 10.8 $nmol \cdot L^{-1}$, respectively, for the (R) and (S)-OME at baseline inhibition and induction, and AUC of 178.62, 1582.69 and 16.35 vs. 234.94, 1746.22 and 21.97 $h \cdot nmol \cdot L^{-1}$, respectively, for the (R) and (S)-OME at baseline inhibition and induction). Moreover, AUC and C_{max} were significantly lower ($p < 0.05$) for *(R)*-OME than for *(S)*-OME at the three sessions.

(S)-OH-OME and *(R)*-OH-OME pharmacokinetic profiles exhibited notable differences in terms of distribution and elimination characteristics. As for the parent compound, no statistically significant differences in terms of T_{max} were observed between *(S)*- and *(R)*-OH-OME.

Regardless of the session considered, the average C_{max} of *(S)*-OH-OME was 7.1-fold lower than that of *(R)*-OH-OME enantiomer (9.8, 3.4 and 8.2-fold, respectively, for control, inhibition and induction sessions). Similarly, *(S)*-OH-OME's AUC was, on average, 7.0-fold lower than *(R)*-OH-OME's AUC (11.0, 3.5 and 8.2-fold, respectively, for control inhibition and induction sessions).

3.2.2 | Effect of the CYP2C19 inhibition and induction

The distribution of the AUC ratios (OH-OME/OME) after OME alone and after its administration with fluvoxamine and voriconazole or rifampicin is presented in Figure 2B.

As expected, at inhibition session, *(S)*- and *(R)*-OME AUC significantly increased (8.1-folds on average), hence decreasing both *(S)*- and *(R)*- AUC ratios and reflecting the inhibition effect.

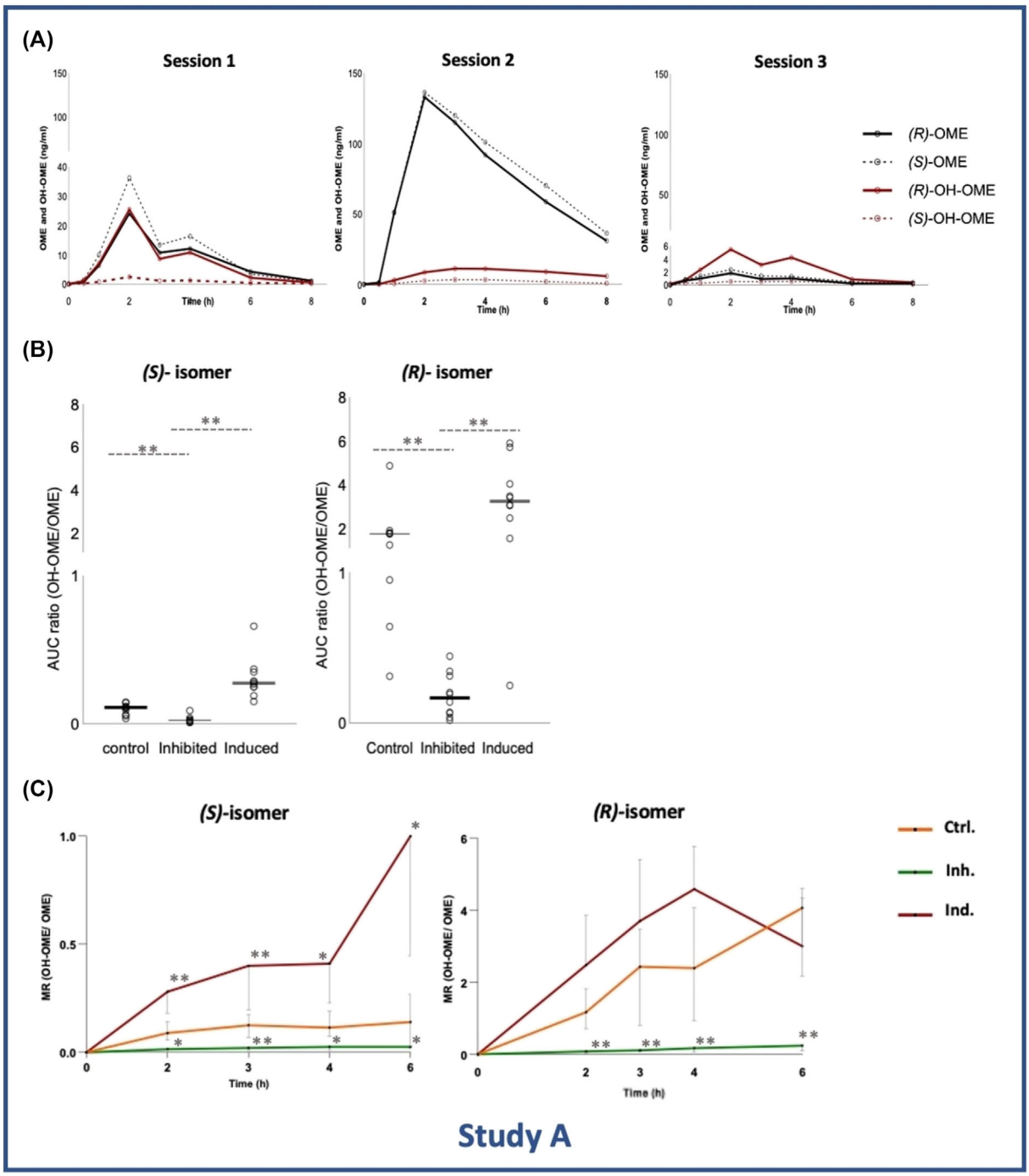

FIGURE 2 Study A pharmacokinetics. (A) (R)- and (S)-OME and 5-hydroxyomeprazole (OH-OME) median plasma concentration time profiles. Session 1 (control session): 5 mg omeprazole (OME) intake alone; Session 2 (voriconazole inhibition session): 5 mg OME intake after inhibition via voriconazole and fluvoxamine; Session 3 (rifampicin induction session): 5 mg OME intake after induction via rifampicin. (B) Area under the curve (AUC) ratio (OH-OME/OME) for (S)- and (R)-isomers (** = $p < 0.01$). (C) Median metabolic ratio (MR)-time profile of (S)-OH-OME/(S)-OME and (R)-OH-OME/(S)-OME (* = $p < 0.05$, ** = $p < 0.01$).

When considering the parent compound enantiomers separately, we note that *(S)*- and *(R)*- AUC are, on average, 7.4 and 8.9-fold higher after inhibition (Table 1). Comparatively, we observe a 2.51 and 0.81-fold increase in *(S)*- and *(R)*-OH-OME AUC after inhibition.

Statistically significant differences regarding the AUC ratio were observed among the three sessions ($p < 0.01$). Median AUC ratios (CI) for *(S)*- and *(R)*- at the control session were 0.11 (0.08; 0.12) and 1.76 (0.93; 2.47), respectively, in contrast to 0.02 (0.01; 0.04) and 0.17 (0.09; 0.27) at the inhibited session.

TABLE 1 Pharmacokinetic parameters of (R) and (S)-OME alone (baseline) or with cytochrome P450 2C19 (CYP2C19) inhibitors (inh.) and inducer (ind.).

	(R)-					*(S)*-				
	Inh.	**Inh. ratio**	**Baseline**	**Ind.**	**Ind. ratio**	**Inh.**	**Inh. ratio**	**Baseline**	**Ind.**	**Ind. ratio**
OME										
AUC_{0-8} ($h \cdot nmol \cdot L^{-1}$)	1582.69	8.86	178.62	16.35	0.09	1746.22	7.43	234.94	21.97	0.09
C_{max} ($nmol \cdot L^{-1}$)	415.34	4.77	87.113	7.99	0.09	446.1	3.53	126.52	10.8	0.09
CL ($nmol \cdot L^{-1}$)	20.35	0.07	278.6	1571.03	5.64	16.428	0.08	215.15	1404.4	6.53
R/S ratio at 2 h	1.03	1.47	0.70	0.76	1.09					
R/S ratio at 4 h	1.10	1.65	0.67	0.76	1.14					
OH-OME										
AUC_{0-8} ($h \cdot nmol \cdot L^{-1}$)	153.24	0.81	190.17	43.68	0.23	43.6	2.51	17.39	6.66	0.38
C_{max} ($nmol \cdot L^{-1}$)	38.79	0.48	80.934	22.04	0.27	11.48	1.38	8.3	2.7	0.33
CL ($nmol \cdot L^{-1}$)	115.05	0.69	166.48	541.39	3.25	712.19	0.44	1619.78	3842.63	2.37
R/S ratio at 2 h	3.37	0.34	9.89	0.10	0.01					
R/S ratio at 4 h	3.38	0.34	9.93	0.14	0.01					
Hydroxylation index	0.12		1.12	4.16		0.03		0.08	0.44	

Note: Hydroxylation index corresponds to the median ratio of the AUC_{0-8} of OH-OME and AUC_{0-8} of OME for each enantiomer and at different sessions.
Abbreviations: AUC, area under the curve; Inh., inhibited; Ind., induced; OH-OME, 5-hydroxyomeprazole; OME, omeprazole.

Pretreatment with rifampicin significantly decreased *(S)*- and *(R)*-OME AUC (10.7-fold on average compared to the control session), increasing the AUC ratio. The average induction impact on *(S)*- and *(R)*-OME enantiomers was of a 10.7 and 10.9-fold decrease for (*S*)- and *(R)*-OME, respectively. When considering the metabolite enantiomers, we note a 2.6- and 4.4-fold decrease for *(S)* and *(R)*-OH-OME AUC, respectively. Median AUC ratios (CI) for *(S)- and (R)*-isomers at the induction session were 0.28 (0.23; 0.4) and 3.26 (2.24; 4.36), respectively.

3.2.3 | MR ratios

The first set (Study A) distributions of the MR OH-OME/OME at times 2, 3, 4 and 6 h for each enantiomer after OME alone and after coadministration with voriconazole and fluvoxamine or rifampicin are presented in Figure 2C.

Statistically significant differences were observed for *(S)*- and *(R)*-enantiomers between the control session and the inhibited one, with $p < 0.05$ at times 2, 4 and 6 h for *(S)*-isomer and $p < 0.01$ at times 3 h for *(S)*- and 2, 3, 4 and 6 h for *(R)*-enantiomer.

Comparatively, statistically significant differences were observed between the control session and the induced one at time points 4 and 6 h ($p < 0.05$) and 2 and 3 h ($p < 0.01$) for the *(S)*-enantiomer. No statistical differences were observed for the *(R)*-enantiomer when considering the induction session. Median (CI) *(R)*- and *(S)*-(OH-OME/OME) MRs over time are presented in Table 2.

3.3 | Study B

3.3.1 | Pharmacokinetic data

Figure 3A shows the pharmacokinetic profiles of *(S)*- and *(R)*-OME and OH-OME when the OME racemic mixture is given alone and when administered with voriconazole and fluvoxamine, respectively (distinctly at Session 2: voriconazole and Session 3: fluvoxamine).

Pharmacokinetic profiles of (S)-OME and (R)-OME were similar in terms of distribution and elimination. No statistically significant differences were observed in the time to maximum concentration (T_{max}: 2 h) between the enantiomers across the study arms. In the control arm, AUC and C_{max} were significantly lower for (R)-OME compared to (S)-OME (AUC: 167 vs. 228 $h \cdot ng \cdot ml^{-1}$; C_{max}: 80 vs. 108 $ng \cdot ml^{-1}$; $p < 0.01$). When co-administered with voriconazole, the differences were not significant (AUC: 593 vs. 715 $h \cdot ng \cdot ml^{-1}$; C_{max}: 161 vs. 193 $ng \cdot ml^{-1}$; $p = 0.73$). Similarly, with fluvoxamine, no significant differences were observed (AUC: 585 vs. 605 $h \cdot ng \cdot ml^{-1}$; C_{max}: 197 vs. 208 $ng \cdot ml^{-1}$; $p = 0.93$).

TABLE 2 OME (R) and (S) median area under the curve (AUC) ratios at the control (ctrl.), inhibited (inh.) and induced session (ind.)—Study A.

	Sesssion 1 (ctrl.)		Sesssion 2 (inh.)		Sesssion 3 (ind.)	
Time	Median (R)-OH-OME/(R)-OME (CI)	Median (S)-OH-OME/(S)-OME (CI)	Median (R)-OH-OME/(R)-OME (CI)	Median (S)-OH-OME/(S)-OME (CI)	Median (R)-OH-OME/(R)-OME (CI)	Median (S)-OH-OME/(S)-OME (CI)
2	1.17 (0.74; 2.19)	0.09 (0.0; 0.36)	0.08 (0.05; 0.13)	0.02 (0; 0.07)	2.48 (1.65; 3.78)	0.28 (0.22; 0.69)
3	2.44 (1.04; 4.59)	0.13 (0.07; 0.23)	0.12 (0.09; 0.19)	0.02 (−0.01; 0.08)	3.71 (2.55; 5.34)	0.4 (0.3; 0.78)
4	2.4 (1.13; 5.67)	0.12 (0.08; 0.19)	0.17 (0.11; 0.28)	0.03 (−0.01; 0.1)	4.6 (3.02; 5.66)	0.41 (0.33; 0.79)
6	4.07 (2.51; 4.22)	0.14 (0.04; 0.41)	0.24 (0.08; 0.68)	0.03 (0.02; 0.05)	3.01 (2.37; 4.36)	1 (0.54; 1.01)

Abbreviations: CI, confidence interval; OH-OME, 5-hydroxyomeprazole; OME, omeprazole.

3.3.2 | Effect of the CYP2C19 inhibition

In comparison to the control session, the median AUC of (R)-OME was 3.8 times higher under voriconazole and 4.3 times higher under fluvoxamine. The median AUC for *(S)*-OME was 3.4 times higher when administered with voriconazole and 3.0 times higher with fluvoxamine. Comparatively, for *(S)*- and *(R)*-OH-OME AUC, no statistically significant difference was observed between inhibition with voriconazole and with fluvoxamine.

In Study B, median AUC ratios (CI) for *(S)*- and *(R)*-OME at the control session were 0.09 (0.05; 0.24) and 2.19 (1.60; 5.15), respectively (Figure 3B).

(S)-isomer median AUC ratio was 0.05 (0.03; 0.06) when co-administered with voriconazole *versus* 0.05 (0.04; 0.06) with fluvoxamine. *(R)*-isomer median AUC ratio was 0.52 (0.33; 1.09) with voriconazole *versus* 0.37 (0.31; 0.59) with fluvoxamine. Both *(S)*- and *(R)*-isomers median AUC ratios were statistically different between the control and voriconazole and between the control and fluvoxamine ($p < 0.01$), but not statistically different when comparing inhibition with voriconazole and fluvoxamine ($p = 0.43$ for *(S)*- and $p = 0.06$ for *(R)*-enantiomer).

3.3.3 | MR ratios

The second data set (Study B) shows similar results to the MR ratios obtained in Study A. Statistically significant differences were observed between the control and fluvoxamine inhibition at times 2 and 3 h ($p < 0.05$) for *(S)*-enantiomer and at 2, 3 and 6 h ($p < 0.01$) for *(R)*-enantiomer (Figure 3C).

3.3.4 | Spearman rank correlation

The Spearman rank correlation coefficients (Ps) between each enantiomer AUC ratio and MRs in Study A at 2, 3, 4 and 6 h after OME administration alone, with voriconazole and fluvoxamine or with rifampicin are shown in Table 3.

These results go together with the MR results observed, except for the *(R)*-enantiomer-induced session where, even though a good correlation between the AUC ratio and the MR is kept, it is difficult to measure CYP2C19 activity as no significant difference was observed.

3.3.5 | Cut-off determination

In this study, we developed specific threshold values using the results from Study A based on the 2-h MR to distinguish individuals with normal CYP2C19 activity from those with decreased (Session 2—Study A) or increased (Session 3—Study A) activity for each enantiomer. These threshold values were established as a preliminary criterion for CYP2C19 phenotyping using *(S)*-enantiomer, thus providing a practical and clinically relevant approach.

Established thresholds for *(S)*- and *(R)*-enantiomers are presented in Table 4 and Figure 4.

When we applied proposed thresholds from Study A to the participants with the NM genotype in Study B, the results fell in line with our expectations. In the control session, where participants received OME alone to simulate individuals with normal activity, the median MR value for the *(S)*-enantiomer closely matched the cut-offs established in Study A, while the median MR value for the *(R)*-enantiomer suggested an RM phenotype.

4 | DISCUSSION AND CONCLUSION

The primary focus of this investigation was to assess how *(S)*- and *(R)*-OME enantiomers pharmacokinetic

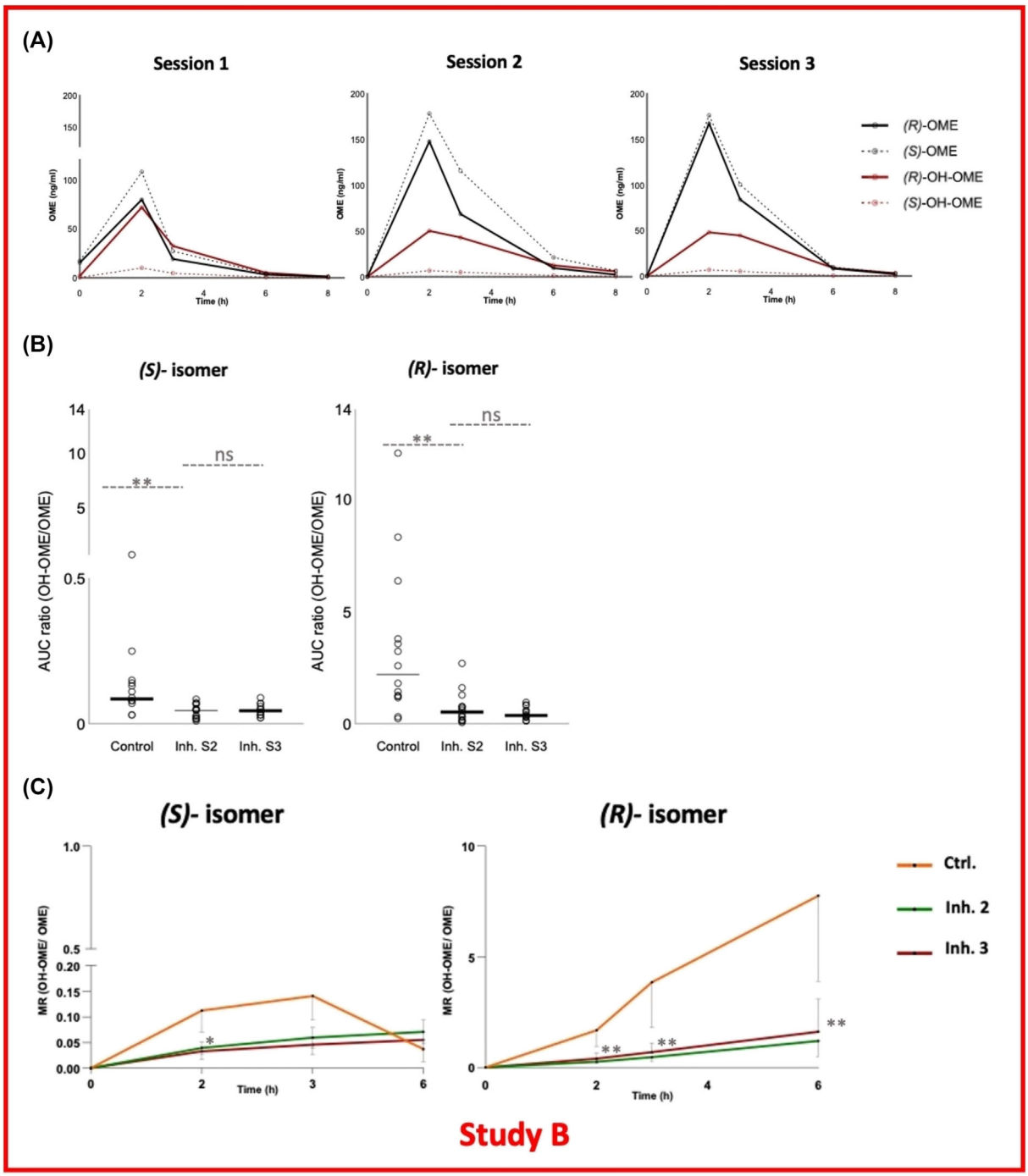

FIGURE 3 Study B pharmacokinetics. (A) (R)- and (S)-OME and 5-hydroxyomeprazole (OH-OME) median plasma concentration time profiles. Session 1 (control session): 10 mg omeprazole (OME) intake alone; Session 2 (voriconazole inhibition session): 10 mg OME intake after inhibition via voriconazole; Session 3 (fluvoxamine inhibition session): 5 mg OME intake after inhibition via fluvoxamine. (B) Area under the curve (AUC) ratio (OH-OME/OME) for (S)- and (R)-isomers (** = $p < 0.01$, ns = not significant). (C) Median metabolic ratio (MR)-time profile (OH-OME/OME) for (S)- and (R)-isomers (* = $p < 0.05$, ** = $p < 0.01$).

properties, in addition to their corresponding hydroxylated metabolites, are affected by the presence of inhibitors (fluvoxamine and voriconazole, both independently and in combination) and an inducer (rifampicin).

The study aimed to evaluate CYP2C19 phenotyping accuracy using a specific metabolic pathway not affected by CYP3A and to evaluate the possibility of phenotyping patients taking esomeprazole as antacid therapy. We utilized pharmacokinetic data from two different sets of experiments, referred to as Study A and Study B.

In Study A, *(S)*- and *(R)*-OME enantiomers pharmacokinetic profiles demonstrated similarities in terms of

TABLE 3 Spearman rank correlations (ρs) between OME (R) and (S) AUClast ratios of metabolite/probe and the metabolic ratios at various time points at different sessions.

		(*S*)-						(*R*)-					
		Inhibition		Baseline		Induction		Inhibition		Baseline		Induction	
Phenotyping index	Sampling time (h)	Ps	*p*-value	Ps	*p*-value	Ps	*p*-value	Ps	*p*-value	Ps	*p*-value	Ps	*p*-value
[OH-OME]/[OME]	2	0.8	0.005	0.97	<0.001	0.78	0.008	0.84	0.002	0.94	<0.001	0.17	0.647
	3	0.86	0.001	0.95	<0.001	0.79	0.006	0.78	0.008	0.92	<0.001	0.52	0.128
	4	0.92	<0.001	1	<0.001	0.74	0.014	0.73	0.016	0.89	0.001	0.79	0.006
	6	−0.15	0.689	0.86	0.001	−0.29	0.415	0.58	0.077	0.88	0.001	−0.04	0.907
	8	−0.34	0.331	0.8	0.005	0	1	0.11	0.772	0.88	0.001	−0.13	0.727

Abbreviations: OH-OME, 5-hydroxyomeprazole; OME, omeprazole.

TABLE 4 Proposed thresholds for cytochrome P450 2C19 (CYP2C19) phenotyping using *(R)*- and *(S)*-omeprazole (OME) isomers.

	(S)-enantiomer	*(R)*-enantiomer
Rapid metabolizer (RM)	0.46 ± 0.38	2.71 ± 1.72
Normal metabolizer (NM)	0.18 ± 0.29	1.47 ± 1.17
Poor metabolizer (PM)	0.04 ± 0.06	0.09 ± 0.06

distribution and elimination characteristics. The same order of concentration magnitude of the parent molecule was measured for the two enantiomers, underlining the administered OME racemic character. Statistically significant differences were observed for *(S)*- and *(R)*-MR between control and modulated sessions (inhibited and induced).

(S)-isomer did show significant differences for both inhibited and induced sessions, contrary to *(R)*-isomer, which did not show any significant difference at the induction session. *(S)*- and *(R)*-OH-OME enantiomers C_{max} and AUC values were significantly different between Sessions 1, 2 and 3. Notably, *(R)*-OH-OME consistently displayed higher AUC values in comparison to *(S)*-OH-OME.

As described previously, upon its rapid absorption, OME undergoes a stereoselective first-pass metabolism mediated by CYP2C19 in favour of *(R)*-enantiomer, explaining the lower OME AUC and the difference in AUC between both enantiomers once they have been metabolized.[10] Moreover, it is of utmost significance to emphasize that the metabolic conversion of the *(R)*-enantiomer occurs via CYP2C19 and CYP3A4, giving rise to the creation of *(R)*-5-OH-OME. Conversely, the primary metabolic process for the *(S)*-enantiomer revolves around CYP2C19, resulting in the formation of 5-O desmethylomeprazole and a minor pathway resulting in the production of *(S)*-5-OH-OME.[11]

Within the framework of this study, our central objective was primarily oriented towards elucidating the hydroxylation pathway for both the *(R)*- and *(S)*-enantiomers. This signifies that we extensively explored the principal route for the *(R)*-isomer while also considering a minor route for the *(S)*-enantiomer.

OME coadministration with CYP2C19 inhibitors, voriconazole and fluvoxamine, led to a significant decrease in OH-OME/OME AUC ratios, reflecting the inhibitory effect on *(S)*- and *(R)*-OME metabolism. It should be noted that, although our study did not specifically focus on the combination of fluvoxamine and voriconazole, this combination resulted in a significant increase in AUC due to the dual inhibition of CYP2C19 by both

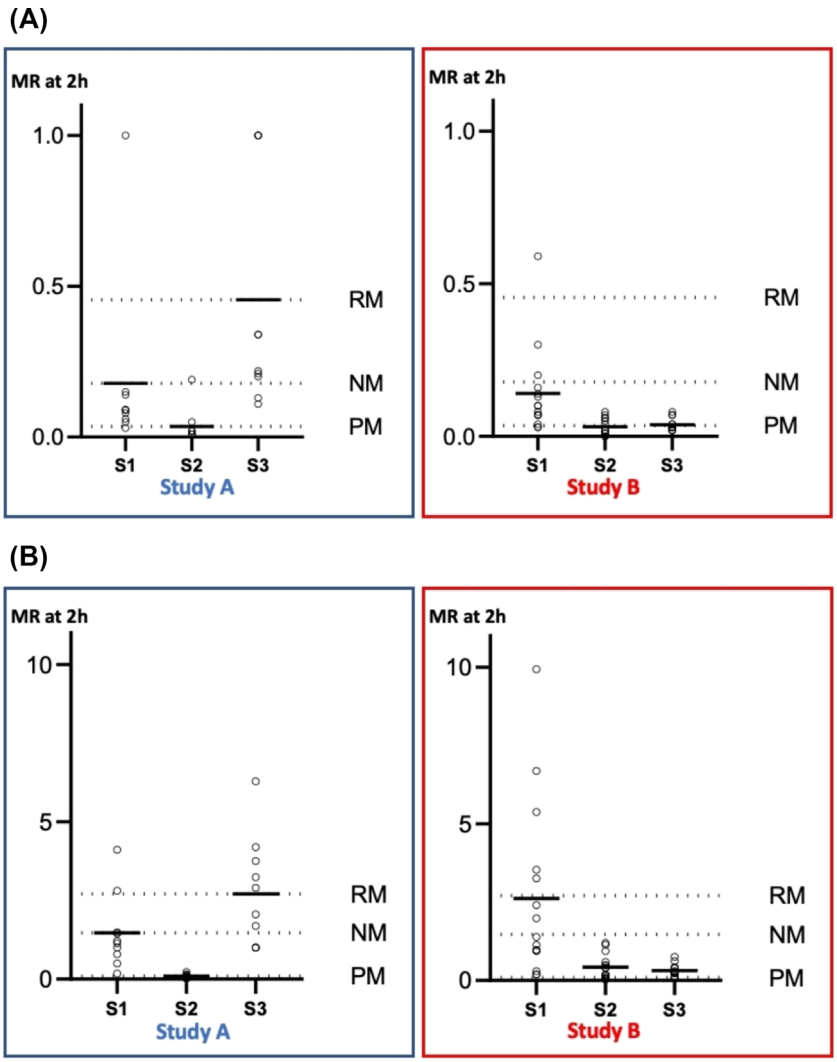

FIGURE 4 (A) (S)-(5-hydroxyomeprazole [OH-OME]/omeprazole [OME]) cut-off determination with Study A and verification with Study B. (B) (R)-(OH-OME/OME) cut-off determination with Study A and verification with Study B.

drugs. This highlights the importance of considering complex interactions when co-administering multiple inhibitors. OME enzyme-catalysed stereoselective metabolism led to slower metabolic clearance of *(S)*-OME compared to the *(R)*-isomer as described by Abelo et al., and inhibition index was 1.9-fold higher on *(R)*-OME when compared to *(S)*-OME.

A great deal of variability in the measured concentrations is observable when the *(R)*-enantiomer is compared with the *(S)* one. However, concomitant use of fluvoxamine (strong CYP2C19 inhibitor) and voriconazole (moderate CY2C19 and strong CY3A4 inhibitor) during the inhibition session (Study A) involves that both CYP2C19 and CYP3A4 pathways were inhibited and could explain this stronger inhibition at the level of the *(R)*-enantiomer.[16]

To evaluate the role of CYP3A4 in the enantiomer metabolism pathway and assess whether it can impact CYP2C19 phenotyping results when *(R)*-enantiomer is considered, we analysed samples from Study B (voriconazole and fluvoxamine administered distinctly at Sessions 2 and 3, respectively).

This second set of data corroborated the trends observed in Study A, with statistically significant differences in AUC values for both enantiomers when CYP2C19 was inhibited moderately (voriconazole) or strongly (fluvoxamine). Median AUC ratios for *(S)*- and *(R)*-isomers were not statistically different between Sessions 2 and 3 ($p = 0.43$ for *(S)* and $p = 0.06$ for *(R)*), indicating that the involvement of CYP3A4 in the metabolism of the *(R)*-enantiomer may have little effect on this pathway. As discussed by Abelo et al., CYP3A4 mainly supports the sulfone metabolite formation from *(R)*-OME, whereas hydroxylation and 5-O-desmethylation are minor pathways via this enzyme.[11]

Pretreatment with rifampicin led to a significant increase in the OH-OME/OME AUC ratios. The induction index was 1.8 for the *(R)*-enantiomer *versus* 2.5-fold for the *(S)*-enantiomer, making it a better candidate for measuring CYP2C19 induction.

At the induction session, rifampicin, as a moderate inducer of CYP2C19 and a strong inducer of CYP3A4, may have had an effect on the CYP2C19 and CYP3A4 pathways in addition to minor ones, introducing variability and impacting statistical significance in our study. Previous studies have reported a similar extent of CYP2C19 induction by rifampicin (4.2- and 2.6-fold).[17]

Spearman correlation results showed that an important correlation existed between AUC_{last} ratio and MRs in plasma even when the CYP activity was modulated. The best correlation was observed at 4 h and was just as significant for *(S)*- as for *(R)*-enantiomer. Previous studies have used a single-point MR at 3 or 4 h as a phenotyping index, which corroborates with our results.[18,19]

In this study, we established specific threshold values using the 2-h MR data from Study A to distinguish individuals with normal, decreased, or increased CYP2C19 activity for both *(R)* and *(S)*-OME enantiomers, providing a practical approach for CYP2C19 phenotyping. These thresholds were confirmed as robust and clinically relevant, particularly for the *(S)*-enantiomer that consistently revealed phenotypic variations when inhibitors were present. The median MR value for the *(R)*-enantiomer suggested an RM phenotype, which was unexpected for NM individuals. This indicates that (*R*)-enantiomer may not be as reliable for phenotyping under these conditions. However, Study B volunteer genotyping was performed investigating only the three most common CYP2C19 variants (CYP2C19*2, *3 and *17), and the wild-type *1 allele was assigned by default when none of the other mutations screened (*2, *3 and *17) were detected. We cannot therefore rule out the possibility that our volunteers have much rarer variants (other than *2, *3 and *17) and that this may have introduced a bias into our study.

An ideal phenotyping probe is a substrate for a transporter or an enzyme that is completely specific. Because currently for CYP2C19 we do not dispose of completely specific probes, it is essential to choose probes with a sufficient degree of selectivity to reflect enzyme activity in various settings. The presented results in this study indicate that we can measure CYP2C19 modulation, hence CYP2C19 phenotype, in a reasonably comparable way between both OME isomers. Because of its lower metabolic clearance, the *(S)*-OME isomer seemed at first sight to be a better candidate for CYP2C19 phenotyping. A slightly more detailed analysis of the pharmacokinetic parameters and the effect of inhibition and induction on each of the enantiomers enabled us to show that CYP3A4 was only minimally involved in the *(R)*-OME isomer metabolism pathway and that inhibition and induction were as measurable for the *(R)*- as for the *(S)*-enantiomer. The difference between both isomers lay in that the measured AUC ratios of the *(R)*-OH-OME were, on average, 10 times higher than those of the *(S)*-OH-OME, which may constitute a limitation from the point of view of the available analytical method limit of quantification. Moreover, the use of metabolic or AUC ratios is advantageous for probes having more than one metabolizing path because they give information on the activity of a single CYP implicated in a specific pathway.[20] In the present investigation, a notable limitation stems from the omission of an exploration into alternative metabolic pathways. Subsequently, conducting additional research on the primary metabolic route for each enantiomer, which involves 5-hydroxylation for the *(R)*-enantiomer and 5-O-desmethylation for the *(S)*-enantiomer, alongside concurrent analysis of the minor pathway, may offer valuable insights. Larger population studies should be conducted to evaluate the distribution of CYP2C19 activity regarding each OME isomer, allowing precise cut-off determination and validation between the different phenotype groups.

In conclusion, this study showed that both *(R)*- and *(S)*-OME enantiomers can be used for CYP2C19 activity assessment. *(R)*-OME enantiomer, induction of CYP2C19 was not as reliable as for (S)-enantiomer. We demonstrated that the *(S)*-enantiomer MR at 2 h and AUC ratio can be used as phenotyping metrics for the CYP2C19 phenotyping. We proposed validated cut-offs for CYP2C19 phenotyping using *(S)*-OME enantiomer, which could facilitate certain clinical situations. The currently available method for CYP2C19 phenotyping requires the administration of OME necessitating, in some cases, the discontinuation of treatment that has already been in place for some time, the most frequent of them being esomeprazole. The cut-offs determined from our study will allow to determine CYP2C19 phenotype in such patients using their own treatment by sampling blood or DBS 2 or 3 h after esomeprazole intake.

Future studies with both single and multiple doses of esomeprazole are necessary to confirm the feasibility and accuracy of this approach. Between January 2013 and April 2014, 56.2% of patients under clopidogrel had a co-prescription of esomeprazole (*(S)*-OME) at Geneva University Hospitals.[21] Once validated in a larger setting, CYP2C19 phenotyping with (*S*)-OME isomers (esomeprazole) could be applied as a tool in clinical practice or specific scenarios.

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CONFLICT OF INTEREST STATEMENT

The other authors have no conflict of interest to declare regarding this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Guengerich FP. Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS j*. 2006;8(1):E101-E111. doi:10.1208/aapsj080112
2. Li-Wan-Po A, Girard T, Farndon P, Cooley C, Lithgow J. Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. *Br J Clin Pharmacol*. 2010;69(3):222-230. doi:10.1111/j.1365-2125.2009.03578.x
3. Hirota T, Eguchi S, Ieiri I. Impact of genetic polymorphisms in *CYP2C9* and *CYP2C19* on the pharmacokinetics of clinically used drugs. *Drug Metab Pharmacokinet*. 2013;28(1):28-37. doi:10.2133/dmpk.DMPK-12-RV-085
4. (DPWG), T.D.P.W.G. *Annotation of DPWG Guideline for clopidogrel and CYP2C19*. [12.10.2023]. Available from: https://www.pharmgkb.org/guidelineAnnotation/PA166104956
5. (CPIC), T.C.P.I.C. *CPIC® Guideline for Clopidogrel and CYP2C19*. [12.10.2023]; Available from: https://cpicpgx.org/guidelines/guideline-for-clopidogrel-and-cyp2c19/
6. Bosilkovska M, Samer CF, Déglon J, et al. Geneva cocktail for cytochrome p450 and P-glycoprotein activity assessment using dried blood spots. *Clin Pharmacol Ther*. 2014;96(3):349-359. doi:10.1038/clpt.2014.83
7. Derungs A, Donzelli M, Berger B, Noppen C, Krähenbühl S, Haschke M. Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the Basel cocktail: a randomized crossover study. *Clin Pharmacokinet*. 2016;55(1):79-91. doi:10.1007/s40262-015-0294-y
8. Frye RF, Matzke GR, Adedoyin A, Porter JA, Branch RA. Validation of the five-drug "Pittsburgh cocktail" approach for assessment of selective regulation of drug-metabolizing enzymes. *Clin Pharmacol Ther*. 1997;62(4):365-376. doi:10.1016/S0009-9236(97)90114-4
9. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet*. 2002;41(12):913-958. doi:10.2165/00003088-200241120-00002
10. Li XQ, Weidolf L, Simonsson R, Andersson TB. Enantiomer/enantiomer interactions between the S- and R- isomers of omeprazole in human cytochrome P450 enzymes: major role of CYP2C19 and CYP3A4. *J Pharmacol Exp Ther*. 2005;315(2):777-787. doi:10.1124/jpet.105.090928
11. Abelö A, Andersson TB, Antonsson M, Naudot AK, Skånberg I, Weidolf L. Stereoselective metabolism of omeprazole by human cytochrome P450 enzymes. *Drug Metab Dispos*. 2000;28(8):966-972.
12. Tveden-Nyborg P, Bergmann TK, Jessen N, Simonsen U, Lykkesfeldt J. BCPT 2023 policy for experimental and clinical studies. *Basic Clin Pharmacol Toxicol*. 2023;133(4):391-396. doi:10.1111/bcpt.13944
13. Abouir K et al. Stereoselective separation of omeprazole and 5-hydroxy-omeprazole using dried plasma spots and a heart-cutting 2D-LC approach for accurate CYP2C19 phenotyping. *J Chromatogr B*. 2023;123962.
14. EMA. *Guideline on bioanalytical method validation*. 2009. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf.
15. David A. Flockhart, *Drug Interactions Flockhart Table* [12.10.2023]; Available from: https://drug-interactions.medicine.iu.edu/MainTable.aspx
16. Kanebratt K, Diczfalusy U, Bäckström T, et al. Cytochrome P450 induction by rifampicin in healthy subjects: determination using the Karolinska cocktail and the endogenous CYP3A4 marker 4β-hydroxycholesterol. *Clin Pharmacol Ther*. 2008;84(5):589-594. doi:10.1038/clpt.2008.132
17. Sim SC, Risinger C, Dahl M-L, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther*. 2006;79(1):103-113. doi:10.1016/j.clpt.2005.10.002
18. Chang M, Dahl ML, Tybring G, Gotharson E, Bertilsson L. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenet Genomics*. 1995;5(6):358-363. doi:10.1097/00008571-199512000-00004
19. Fernando de Andrés ST, Bovera M, Fariñas H, Terán E, Llerena A. Multiplex phenotyping for systems medicine: a one-point optimized practical sampling strategy for simultaneous estimation of CYP1A2, CYP2C9, CYP2C19, and CYP2D6 activities using a cocktail approach. *OMICS: J Integr Biol*. 2016;20(2):88-96.
20. Vernaz N, Rollason V, Adlere L, et al. Snapshot of the prescribing practice for the clopidogrel and esomeprazole coprescription and cost evaluation of the application guidelines. *Pharmacol Res Perspect*. 2016;4(3):e00234. doi:10.1002/prp2.234

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