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Identification of the Acid Induced Degradation Products of Omeprazole and 5-hydroxyomeprazole by High Resolution Mass Spectrometry (HRMS).

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Rationale

Omeprazole is used to treat gastric disorders and is one of the most commonly consumed drugs in the western world. It forms several metabolites but is mostly excreted unchanged and as 5-hydroxyomeprazole. Since omeprazole is widely prescribed, its excretion from the body has a potential environmental effect. After excretion it will enter the wastewater system and if not adequately removed during wastewater treatment will be discharged into rivers in the wastewater effluent. It is important to consider not only the parent drug but also the main metabolite (5-hydroxyomeprazole) and their degradation products to fully understand the fate of this drug during wastewater treatment. In order to do this potential degradation products need to be determined.

Methods

In this study, acid was used to artificially accelerate degradation of omeprazole and 5-hydroxyomeprazole. A Thermo Scientific Q-Exactive Orbitrap mass spectrometer with electrospray ion source was used to determine precursor and product ion data for the degradation products.

Results

Both starting materials quickly degrade under acidic conditions and the main degradation product formed in each case was a re-arranged monomer. Other products formed were doubly and singly charged dimer ions with varying numbers of sulphur atoms in the dimer bridge. Careful interpretation of the accurate mass, isotope pattern, isotope abundance and product ion spectra were used to interpret the data.

Conclusion

On comparing the results from omeprazole and 5-hydroxyomeprazole the resultant degradants were analogous to each other, differing only by an oxygen atom. This investigation determined the degradation products of omeprazole and 5-hydroxyomeprazole and proposed structures based on the accurate mass and isotope information. The product ions from the degradation products are also reported.

Introduction.

Omeprazole (Figure 1 (A)) is a proton pump inhibitor used in the treatment of gastric disorders and was first marketed in 1989 by Astra Zeneca. It is the most commonly prescribed proton pump inhibitor in the western world[1]. In 2015, the highest number of community prescriptions in Scotland were for omeprazole, amounting to 3783 kg (including esomeprazole) of the active ingredient [2]. Omeprazole is formulated with an enteric coating to prevent degradation in the stomach and ensure the drug reaches the parietal cell intact. Once the drug is absorbed through the intestine and into the parietal cell, it is the action of the acid excreted in the cell on omeprazole which causes the molecule to re-arrange[3]. The re-arranged molecule reacts with the thiol on the acid-producing enzyme[4] blocking further acid production (Scheme 1). Since the acid production is now blocked, the excess omeprazole cannot re-arrange and is metabolised prior to excretion. Several metabolites are formed however, according to Lagerstrom[5] the main metabolite detected in urine samples is 5-hydroxyomeprazole (Figure 2(A)) and small amounts of omeprazole and omeprazole sulphone.

There is currently much interest concerning micro-pollutants in wastewater[6–15] though little consideration is given to the metabolites or degradation products. In view of omeprazole's widespread use, the resultant degradants for omeprazole and 5-hydroxyomeprazole are of great interest as potential environmental pollutants. In order to accelerate the degradation and determine the resultant products, omeprazole and 5-hydroxyomeprazole were treated with acid and the degradation followed using accurate mass data from a Thermo Scientific Q-Exactive Orbitrap[16,17] instrument which is a high resolution mass spectrometer (HRMS). HRMS instruments are capable of determining a measured *m/z* with a high degree of certainty, increasing confidence in compound identification and elemental composition assignment. The Thermo Scientific Q-Exactive Orbitrap instrument has the capability to obtain product ion data (MS^2) from the precursor ion giving further valuable structural information.

Experimental

Chemicals and Materials

Optima LCMS grade acetonitrile and formic acid (98%) were purchased from Fisher Scientific (Loughborough, UK), omeprazole and 5-hydroxyomeprazole from Fluka (Darmstadt, Germany) and ammonium formate solution (10M in water) BioUltra, from Sigma-Aldrich (Darmstadt, Germany). An Elga (High Wycombe, UK) Purelab Classic water deioniser was used to provide water at 18 M Ω purity.

LCMS Conditions

The mass spectrometer was a Thermo Scientific Q-Exactive Orbitrap mass spectrometer, fitted with a Dionex Ultimate 3000 RS Pump, Dionex Ultimate 3000 RS autosampler (temperature controlled at 10^{o}C) and Dionex Ultimate 3000 RS column compartment (temperature controlled at 30^{o}C) (All from Thermo Fisher Scientific, Hemel Hempstead, England).

The mass spectrometer was fitted with an electrospray ion source (ESI) operated in positive ion mode. The nitrogen sheath and auxiliary gas were set at 45 and 10 arbitary units. The spray voltage was +3.5 kV and the ion source temperature 300^{o}C.

The full MS experiment scan range was *m/z* = 100 to 900, with the resolution set at 35000. The product ion experiment (MS^2) was conducted using a mass resolution of 17500. The isolation window for the product ion experiment was 4.0 u with a normalised collision energy (NCE) of 40 eV.

A Waters (Elstree, UK) Atlantis dC18 chromatography column 150 × 2.1 mm, particle size 3 µm was used for the chromatographic separation.
The organic solvent was CH_3CN (A) and the aqueous (B) was18 M Ω purity water containing 10 mmol ammonium formate adjusted to pH 3.5 with formic acid. A gradient elution technique was used. The initial conditions were 99% B for 1 minute, dropping to 60% B over 11 minutes then 1% B over 6 minutes. The gradient was maintained at 1% B before returning to 99% B over 1 minute and equilibrating for a further 7 minutes. The flow rate was 0.3 $mL.min^{-1}$.
The software was Tracefinder to operate the chromatography and mass spectrometry system and Xcalibur for MS interpretation.
Prior to commencement of the analysis the instrument was calibrated in positive ion mode using Pierce LTQ Velos ESI Positive ion calibration solution, ex Fisher Scientific (Loughborough, England).

Acid Treatment of Omeprazole and 5-hydroxyomeprazole

Separate solutions of omeprazole and 5-hydroxyomeprazole were prepared at a concentration of 5000 $ng.mL^{-1}$ in CH_3CN/H_2O 20/80. These solutions (1 mL) were treated with 100 µL of 0.2 M HCl in water with mixing. Samples were placed in the autosampler at 10^oC and analysed every 30 minutes up to 48 hours after treatment with acid. The 20 hour sample was selected to determine the degradants as the retention time and area of the peaks in the chromatogram were consistent and was deemed the end of the degradation as a steady state was reached.

Results and Discussion

Chromatography and Product Ion Data of Omeprazole and 5-hydroxyomeprazole

Using the conditions described previously, the retention time of omeprazole (Figure 1(A)) was 13.1 minutes (*m/z* =346.1266) and 5-hydroxyomeprazole (Figure 2(A)) was 11.0 minutes (*m/z* = 362.1167).
Having determined the retention time and precursor ion for both starting materials the product ion spectrum was also acquired using a collision energy of 40 eV. The measured *m/z* of the product ions formed from both omeprazole and 5-hydroxyomeprazole are described in Table 2 and a formula for each product ion is proposed. The mass measurement error was calculated from the experimental and theoretical data using Equation 1[18].
The product ions for omeprazole were in keeping with the literature values[19,20]. On comparing the product ions from omeprazole and 5-hydroxyomeprazole (Table 1) they were analogous, differing only by an O atom, with the exception of *m/z* = 136.0758 and 149.0709 which are common to both precursor ions. The common product ions conform to the benzimidazole part of the molecule which is identical for both precursor ions. The mass measurement error for omeprazole and 5-hydroxyomeprazole product ions are < 3 ppm and are well within the recommended minimum of <10 ppm[18,21] required for product ions.

Chromatography and Accurate Mass of the Degradants in Acid Treated Omeprazole and 5-hydroxyomeprazole

The instability of omeprazole and 5-hydroxyomeprazole was exploited and both were separately treated with 0.2 M HCl to accelerate their degradation. The chromatograms acquired, 20 hours after acid addition, were selected for elucidation. Their total ion chromatograms (TIC) in positive ion mode (*m/z* = 100 – 900) are shown in Figures 1(B) for omeprazole and 2(B) for 5-hydroxyomeprazole.
The peak eluting at 13.1 minutes in the degraded omeprazole sample (Figure 1(B) has the same retention time as omeprazole. However, the isotope pattern for omeprazole comprised

of ions with *m/z* = 346.1219, 347.1252, 348.1176 and 349.1210. The degradation product at the same retention time has an isotope pattern with *m/z* = 345.1053, 345.6070, 346.1033 and 346.6049 which is consistent with a doubly charged dimer ion, proving it is not omeprazole (Figure 3). A false positive for omeprazole could result if the dimer isotope (*m/z* = 346.1033) is mistaken for omeprazole either by using an instrument of lower resolution or poor interpretation of the data.

Rationale for Interpretation of MS Data

ChemCalc software[22] was used to generate possible formulae from the measured accurate mass of the unknown degradants. The degradants in the experiment are not totally unknown as the molecular structure of the starting material is already established[21]. The closest theoretical and measured accurate mass were compared taking in to consideration the atoms constituting the parent molecule. For example for omeprazole (Figure 1(A)) and 5-hydroxyomeprazole (Figure 2 (A)) the imidazole and pyridine nitrogen atoms are likely to remain intact therefore the degradation products are likely to contain nitrogen atoms in multiples of three. Once the measured accurate mass of the precursor ions were determined, the product ion spectrum was acquired to obtain further structural information about the unknown degradants. The calculated mass measurement error for all the proposed formulae was < 2.1ppm (Table 2) which far surpasses the FDA guidelines[21] of <5ppm for precursor ions. Based on the re-arrangement described by Brandstrom and Lindberg[23–26] the unknown degradants can be determined and structures proposed. Consideration of the data indicates that monomer and dimer degradation products of omeprazole and 5-hydroxyomerazole formed and consist of singly and doubly charged ions.

A+1 and A+2 Isotope Information

The isotope pattern of an ion can give valuable information about the formula. (The most abundant isotope is designated A. The contribution from the ^{13}C, ^{15}N, ^{17}O and ^{33}S isotopes is designated A+1 and the ^{34}S cluster, with a lesser contribution from the other element isotopes, as A+2). The relative abundance of the A+1 isotope when compared to the main isotope can give an estimation of the number of carbon atoms in a molecule[27,28]. Measuring the abundance of the A+1 isotope to determine the number of C atoms in the molecule can prove inaccurate especially using ion trap instruments. Interferences can occur using ion trap instruments if too many ions are allowed into the trap at the same time. To limit the ion population inside the trap, there is a cut off for those of lower abundance such as the A+1 isotopes. Therefore, in this case it is used to estimate the number of C atoms and distinguish between monomer and dimer ions[29].

The S atom also has a distinctive isotope pattern which aids interpretation of the HRMS data. The ^{34}S isotope has an abundance of 4.52%, which is greater than the ^{33}S isotope with an abundance of 0.8%. Omeprazole and 5-hydroxyomeprazole contain S and display this distinctive A+2 isotope pattern due mainly to sulphur.

The experimental and theoretical isotope patterns are also compared including the mass shift between A and A+1 isotopes to establish if the ions are singly or doubly charged. In doubly charged ions the A+1 isotope differs by 0.5 u (nominal mass) instead of 1 u (nominal mass). Using the data described in Table 2, 5OH5 and 5OH3A both of which have very similar *m/z* are compared. 5OH5 has *m/z* = 346.1223 and the A+1 isotope is *m/z* = 347.1255. The abundance of the A+1 isotope is approximately 15% and the mass shift is 1 u (nominal mass). This ion is therefore singly charged and contains the correct A+1 isotope abundance for a molecule containing around 15 C atoms and in this case is consistent with a monomer ion. 5OH3A has *m/z* = 345.1147 and the A+1 isotope is *m/z* = 345.6164. The abundance of the A+1 isotope this time is approximately 32% and the mass shift is 0.5 u (nominal mass).

Therefore this ion is doubly charged, contains over 30 C atoms and is consistent with a dimer ion.
An unknown ion with *m/z* = 455.1419 was detected in the omeprazole degradation experiment and corresponded to a possible formula of $C_{17}H_{33}N_3O_3S_4$. The A+1 isotope ratio was approximately 20% and the mass error was calculated as 4.3943 ppm which fit for the proposed formula. However, the A+2 isotope ratio was only 1.2% and does not conform to a substance containing 4 S atoms. This illustrates the importance of interpreting all of the isotope information.
Using the measured accurate mass and isotope information structures for the most common degradants of omeprazole and 5-hydroxyomeprazole have been proposed and are described in Table 2.
The similarity of the degradation products formed when omeprazole and 5-hydroxyomeprazole were treated with 0.2 M HCL was notable. The precursor ion data for the acid degraded omeprazole and 5-hydroxyomeprazole differ by 1 O atom for monomer ions and 2 O atoms for dimer ions. For both omeprazole and 5-hydroxyomeprazole the main degradation product based on peak area was a re-arranged monomer (Table 2, OMEP5 and 5OH5). Another monomer was also detected from both starting materials with no S atom (Table 2, OMEP1 and 5OH1), the low abundance of the A+2 isotope (1.5% and 1%) confirmed the absence of S atoms in the ion. Analogous singly and doubly charged dimer ions from both starting materials formed with between 1 and 3 S atoms in the bridge.
The dimer ions with 2 S atoms in the bridge are easier to explain as these could be formed from 2 re-arranged molecules combining. However both starting materials produced a dimer ion with 3 S atoms in the bridge. One S atom has an exact mass of 31.9715 u and 2 O atoms 31.9893 u. Since these values are so close it was necessary to confirm the ion contained 3 S atoms with 4 O atoms and not 2 S atoms with 6 O atoms. For example OMEP3B has an accurate mass of 689.2031. The formula for this was proposed as $C_{34}H_{37}N_6O_4S_3^+$ with an exact mass of 689.2033. If 1 S atom was swapped for 2 O atoms the formula would be $C_{34}H_{37}N_6O_6S_2^+$ with an exact mass of 689.22105. The calculated mass measurement error would be -0.2902 ppm and -26.0439 ppm respectively, hence it is much more likely to be the formula with 3 S atoms. The abundance of the A+2 isotope is 15% which is in agreement with a molecule containing 3 S atoms. Structures OMEP1 and 5OH1 (Table 2) have both lost the S atom and this may be the source of the third S atom in the bridge.
The multiple sulphur atoms in the bridge was unexpected. However, it can be explained given the affinity of the re-arranged omeprazole for the thiol on the target enzyme[3,23–26]. The proposed mechanism for dimer formation on acid treatment of omeprazole and 5-hydroxyomeprazole is presented in Scheme 1.

Product Ions of Omeprazole and 5-hydroxyomeprazole Degradation Products
It has been established that the precursor ions for the degradation products of omeprazole and 5-hydroxyomeprazole differ by either 1 or 2 O atoms for monomer and dimer ions respectively. The product ions for the degradation products of omeprazole and 5-hydroxyomeprazole were acquired to determine if they too were analogous and had a mass shift equivalent to an O atom. These are reported in Table 3 and 4 respectively.

Product Ions of Monomer Degradants
Both omeprazole and 5-hydroxyomeprazole re-arranged to a main monomer degradation product (OMEP5 and 5OH5). The precursor and product ions had a mass shift equivalent to an oxygen atom, which is the difference between the two substrate molecules (Tables 3 and 4).

The product ions *m/z* = 297.1468 and *m/z* = 313.1413 (Tables 4 and 5) are unusual as they are radical cations containing an odd number of electrons. This was also observed for one of the product ions for omeprazole (*m/z* = 151.0992) and 5-hydroxyomeprazole (*m/z* = 167.0940) described in Table 1. The even electron rule states "even electron ions tend to form even electron product ions whereas odd electron ions tend to dissociate to form either odd or even electron ions" [30]. Therefore the radical cations are in violation of this rule as they are from an even electron precursor ion. From the measured accurate mass data no other formula could be proposed except the radical cation. Violations to the even electron rule are known[30–32] especially in highly conjugated systems like omeprazole and 5-hydroxyomeprazole.

Summary of Common Product Ions for Dimer Degradants in Acid Treated Omeprazole
For the omeprazole degraded samples, the singly charged dimer precursor ions gave the same product ions regardless of the number of sulphur atoms in the sulphur bridge (Table 3). This was also observed for the singly charged dimer ions from 5-hydroxyomeprazole degradants which also gave the same product ions as each other but differed from omeprazole by an O atom.
The product ion *m/z* = 149.0710 is common to both substrates which is indicative of the benzimidazole part of the molecule (Tables 3 and 4). This product ion is also observed in the product ion spectrum of omeprazole and 5-hydroxyomeprazole (Table 1) having no acid treatment. On comparing the product ion spectra of the degradation products, the product ions generated from the singly charged dimer ions are slightly different from the doubly charged equivalent, this is regardless of the number of sulphur atoms linking the dimers (Tables 3 and 4). The doubly charged dimer ions from both substrates did not yield product ions at *m/z* = 328.1108 and 295.1312 for omeprazole and 344.1058 and 311.1258 for 5-hydroxyomeprazole. However the others described above were generated.

Conclusion

Although HRMS cannot unequivocally characterise the molecular structure of the degradation products of omeprazole and 5-hydroxyomeprazole in acid, accurate information about the elemental composition has been obtained. Coupled with the re-arrangement reported by Brandstrom credible molecular structures for the degradation products have been proposed for the first time, from the HRMS data as reported in this paper. This will in turn aid their identification in waste water and environmental locations.

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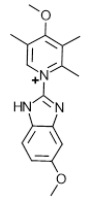
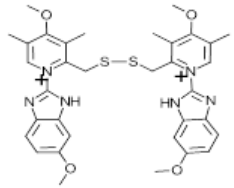
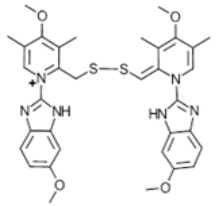
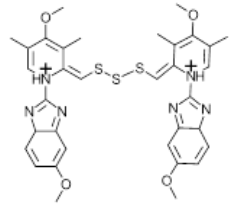
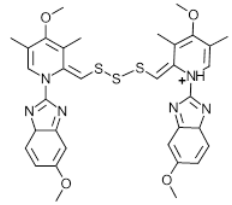
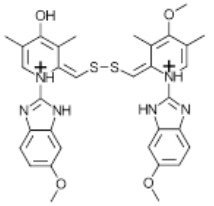
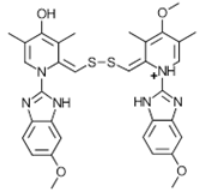
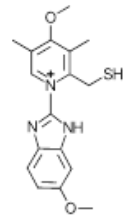
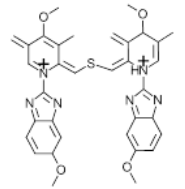
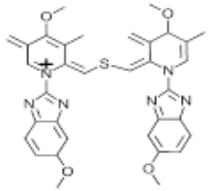
Table 1. Predicted formulae for omeprazole and 5-hydroxyomeprazole product ions based on accurate mass data. The mass measurement error for each product ion has been calculated (Equation 1) and is less than 3 ppm.

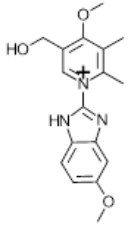
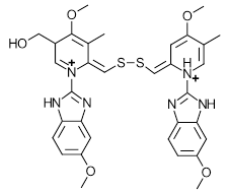
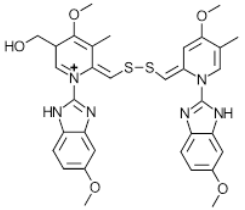
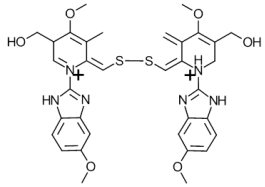
Omeprazole Product Ions				5-Hydroxyomeprazole Product Ions			
Formula	**Exact Mass**	**Accurate Mass**	**Mass Accuracy (ppm)**	**Formula**	**Exact Mass**	**Accurate Mass**	**Mass Accuracy (ppm)**
$C_9H_{12}NO_2S^+$	198.05833	198.0581	-1.16	$C_9H_{12}NSO_3^+$	214.05324	214.0531	-0.65
$C_9H_{10}NOS^+$	180.04776	180.0477	-0.33	$C_9H_{10}NSO_2^+$	196.04268	196.0425	-0.92
$C_9H_{14}NO_2^+$	168.10191	168.1019	-0.06	$C_9H_{13}NO_2^{+\bullet}$	167.09408	167.0940	-0.48
$C_9H_{13}NO^{+\bullet}$	151.09917	151.0992	0.20	$C_8H_{10}NO_2^+$	152.07061	152.0705	-0.72
$C_8H_9N_2O^+$	149.07094	149.0707	-1.61	$C_8H_9N_2O^+$	149.07094	149.0709	-0.27
$C_8H_{10}NO^+$	136.07569	136.0757	0.07	$C_8H_{10}NO^+$	136.07569	136.0758	0.81

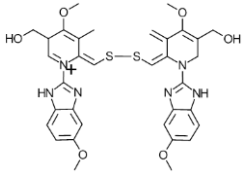
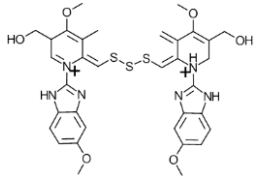
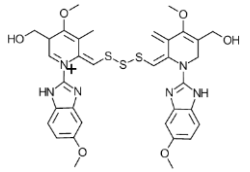
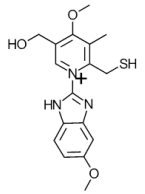
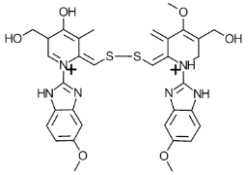
Table 2. Extracted ion data (positive ion mode) for the main precursor ions for acid degraded omeprazole and 5-hydroxyomeprazole. The accurate mass and exact mass for the A, A+1 and A+2 isotopes are described and the mass measurement error of the isotopes calculated. The approximate relative abundance of the A+1 and A+2 isotopes are also reported. Based on the molecular structures of the starting material, the formula determined from the accurate mass and the re-arrangement described by Brandstrom[20, 21, 22, 23, 24], structures have been proposed.

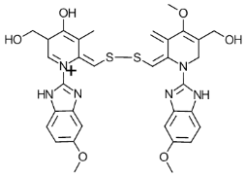
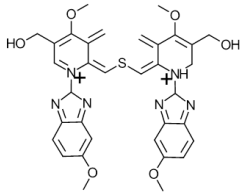
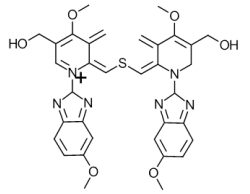
Retention Time (Minutes)	Accurate Mass (Isotopes) A	Accurate Mass (Isotopes) A+1	Approximate Relative Abundance of A+1 Isotope	Exact Mass (Isotopes) A	Exact Mass (Isotopes) A+1	Proposed Formula	Mass Measurement Error M (ppm)	Accurate Mass and Approximate Relative Abundance of A+2 Isotope Cluster[#]	Proposed Structure
Omeprazole and Degradants									
10.8 OMEP1	298.1552	299.1586	16%	298.1550	299.1584	$C_{17}H_{20}N_3O_2^+$	0.6708	300.1622 1.5%	
12.2 OMEP2A	329.1199	329.6216	27%	329.1192	329.6209	$C_{34}H_{37}N_6O_4S_2^{2+}$	2.1269	330.1178 and 330.1231 9%	
OMEP2B	657.2312	658.2349	37%	657.2312	658.2346	$C_{34}H_{37}N_6O_4S_2^+$	0.0000	659.2262 and 659.2387 11%	

13.1 OMEP3A	345.1058	345.6076	35%	345.1053	345.6070	$C_{34}H_{37}N_6O_4S_3^{2+}$	1.4488	346.1040 and 346.1092 14%	
OMEP3B	689.2031	690.2068	37%	689.2033	609.2066	$C_{34}H_{37}N_6O_4S_3^{+}$	-0.2902	691.1987 and 691.2100 15%	
13.8 OMEP4A	322.1118	322.6136	31%	322.1114	322.6131	$C_{33}H_{35}N_6O_4S_2^{2+}$	1.2418	323.1098 and 323.1152 12%	
OMEP4B	643.2155	644.2190	35%	643.2156	644.2189	$C_{33}H_{35}N_6O_4S_2^{+}$	-0.1555	645.2112 and 645.2219 12%	

14.4 OMEP5	330.1274	331.1306	14%	330.1271	331.1304	$C_{17}H_{20}N_3O_2S^+$	0.9087	332.1230 and 332.1342 3.28%	
15.8 OMEP6A	312.1257	312.6275	36%	312.1254	312.6271	$C_{34}H_{35}N_6O_4S^{2+}$	0.9612	313.1238 and 313.1290 7%	
OMEP6B	623.2436	624.2472	37%	623.2435	624.2469	$C_{34}H_{35}N_6O_4S^+$	0.1605	625.2389 and 625.2510 9%	
17.2/17.4 OMEP7	312.0803	313.0836	15%	312.0801	313.0835	$C_{16}H_{14}N_3O_2S^+$	0.6409	314.0762 and 314.0873 5%	Not identified

5-hydroxyomeprazole and Degradants									
9.76 5OH1	314.1502	315.1534	15%	314.1499	315.1533	$C_{17}H_{20}N_3O_3^+$	0.9550	316.157 1%	
10.5 5OH2A	330.1093	330.6110	31%	330.1089	330.6106	$C_{33}H_{36}N_6O_5S_2^{2+}$	1.2117	331.1071 and 331.1124 10%	
5OH2B	659.2104	660.2140	37%	659.2105	660.2138	$C_{33}H_{35}N_6O_5S_2^+$	-0.1517	661.2046 and 661.2167 11%	
10.9 5OH3A	345.1147	345.6165	32%	345.1142	345.6158	$C_{34}H_{37}N_6O_6S_2^{2+}$	1.4488	346.1127 and 346.1179 8%	

5OH3B	689.2209	690.2246	37%	689.2211	690.2244	$C_{34}H_{37}N_6O_6S_2^+$	-0.2902	691.2149 and 691.2276 11%	
11.2 5OH4A	361.1005	361.6022	36%	361.1002	361.6019	$C_{34}H_{38}N_6O_6S_3^{2+}$	0.8308	362.0984 and 362.1036 16%	
5OH4B	721.1931	722.1964	36%	721.1931	722.1965	$C_{34}H_{37}N_6O_6S_3^+$	0.0000	723.1868 and 723.2000 14%	
11.7 5OH5	346.1223	347.1258	15%	346.1220	347.1253	$C_{17}H_{20}N_3O_3S^+$	0.8667	348.1180 and 348.1292 4%	
12.3 5OH6A	338.1066	338.6086	31%	338.1063	338.6080	$C_{33}H_{36}N_6O_6S_2^{2+}$	0.8873	339.1046 and 339.1098 13%	

5OH6B	675.2052	676.2087	34%	675.2054	676.2088	$C_{33}H_{35}N_6O_6S_2^+$	-0.2962	677.2005 and 677.2112 12%	
14 (5OH7A)	328.1205	328.6223	32%	328.1203	328.6220	$C_{34}H_{36}N_6O_6S^{2+}$	0.6095	329.1239 and 329.1187 10%	
(5OH7B)	655.2331	656.2367	36%	655.2333	656.2367	$C_{34}H_{35}N_6O_6S^+$	-0.3052	657.2407 and 656.2288 8%	
15.7/15.9 (5OH8)	328.0753	329.0786	15%	328.0750	329.0784	$C_{16}H_{14}N_3O_3S^+$	0.9144	330.0711 and 330.0823 4%	Not identified

The A+2 isotope cluster could not be completely resolved therefore, the accurate mass at the apex of the unresolved isotopes are reported and the % abundance combined.

Table 3. Product ions from the degradation products of omeprazole.

Omeprazole								
	Sulphur Atoms in Bridge	**Accurate Mass of Precursor Ion**	**Product Ions (*m/z*)**					
OMEP5	Monomer	330.1266^{+}	297.1468				182.063	149.0706
OMEP2B	2	657.2312^{+}		328.1106	295.1309	282.1237	182.0629	149.0706
OMEP4B	2	643.2155^{+}		328.1108	295.1312	282.1234	182.0631	149.0707
OMEP3B	3	689.2031^{+}		328.1108	295.1311	282.1233	182.063	149.0706
OMEP3A	3	345.1058^{2+}				282.1233	182.0609	149.0706
OMEP2A	2	329.1199^{2+}				282.1232	182.063	149.0706

Table 4. Product ions from the degradation products of 5-hydroxyomeprazole.

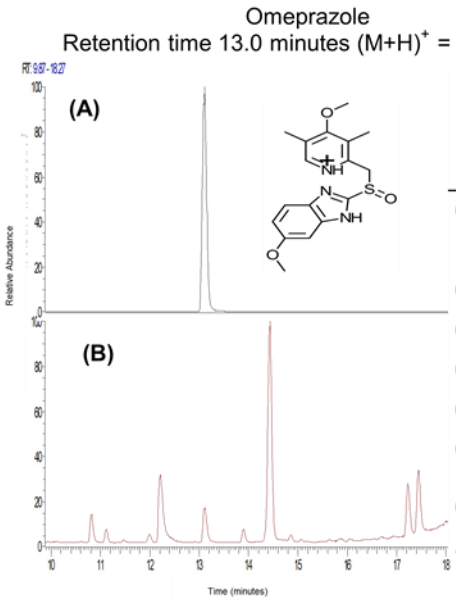
5-Hydroxyomeprazole								
	Sulphur Atoms in Bridge	**Accurate Mass of Precursor Ion**	**Product Ions (*m/z*)**					
5OH5	Monomer	346.1223^{+}	313.1413				198.0578	149.0706
5OH7B	1	655.2331^{+}		344.1058	311.1258	298.1181		149.0708
5OH3B	2	689.2209^{+}		344.1058	311.1259	298.1181	198.0579	149.0706
5OH2A	2	330.1093^{2+}				298.1182	198.0578	
5OH3A	2	345.1147^{2+}				298.1182	198.0579	
5OH7A	1	328.1205^{2+}				298.1181		149.0706

Equation 1:

$$\textbf{Mass measurement error (ppm)} = \frac{\textbf{Accurate mass} - \textbf{Exact mass}}{\textbf{Exact mass}} \times 1000000$$

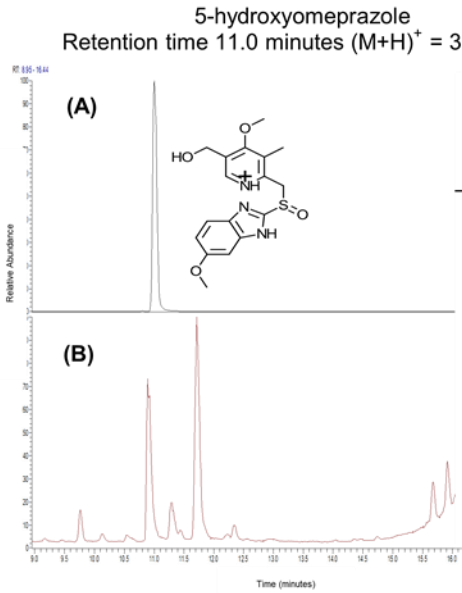
Accurate mass = Measured mass.

Exact mass = Calculated mass from a known elemental formula.



Retention Time (minutes)		$(M+H)^+$	$(M+H)^{2+}$
OMEP1	10.8	298.1553	
Unknown	11.1	455.1421	
OMEP2	12.2	657.2311	329.1199
OMEP3	13.1	689.2023	345.1053
OMEP4	13.8	643.2156	322.1119
OMEP5	14.4	330.1273	
OMEP6	15.8	623.2436	312.1257
OMEP7	17.2	312.0800	
	17.4	312.0800	

Figure 1(A). Molecular structure and extracted ion chromatogram of omeprazole ($(M+H)^+$ = 346.1266). (B) Chromatogram of acid treated omeprazole showing retention time and measured accurate mass of degradants. (Positive ion mode using electrospray ionisation, scan range *m/z* = 100 – 900).



	Retention Time (minutes)	$(M+H)^+$	$(M+H)^{2+}$
5OH1	9.7	314.1502	
5OH2	10.5	659.2104	330.1093
5OH3	10.9	689.2209	354.1147
5OH4	11.2	721.1931	361.1005
5OH5	11.7	346.1223	
5OH6	12.3	675.2052	338.1066
5OH7	14	655.2331	328.1205
5OH8	15.7	328.0753	
	15.9	328.0753	

Figure 2(A). Molecular structure and extracted ion chromatogram of 5-hydroxyomeprazole ($(M+H)^+$ = 362.1167). (B). Chromatogram of acid treated 5-hydroxyomeprazole showing retention time and measured accurate mass of degradants. (Positive ion mode using electrospray ionisation, scan range *m/z* = 100 – 900).

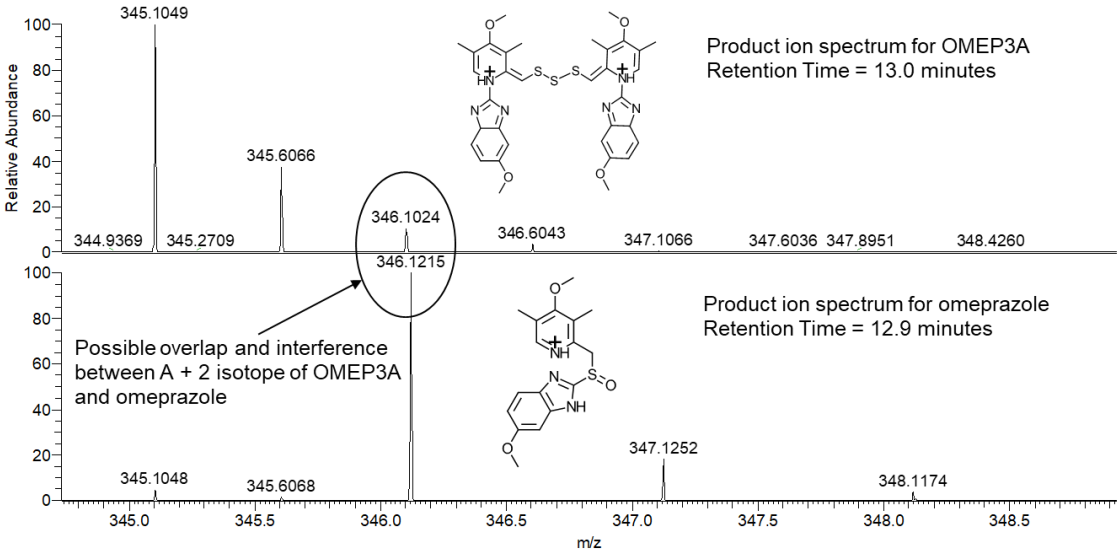
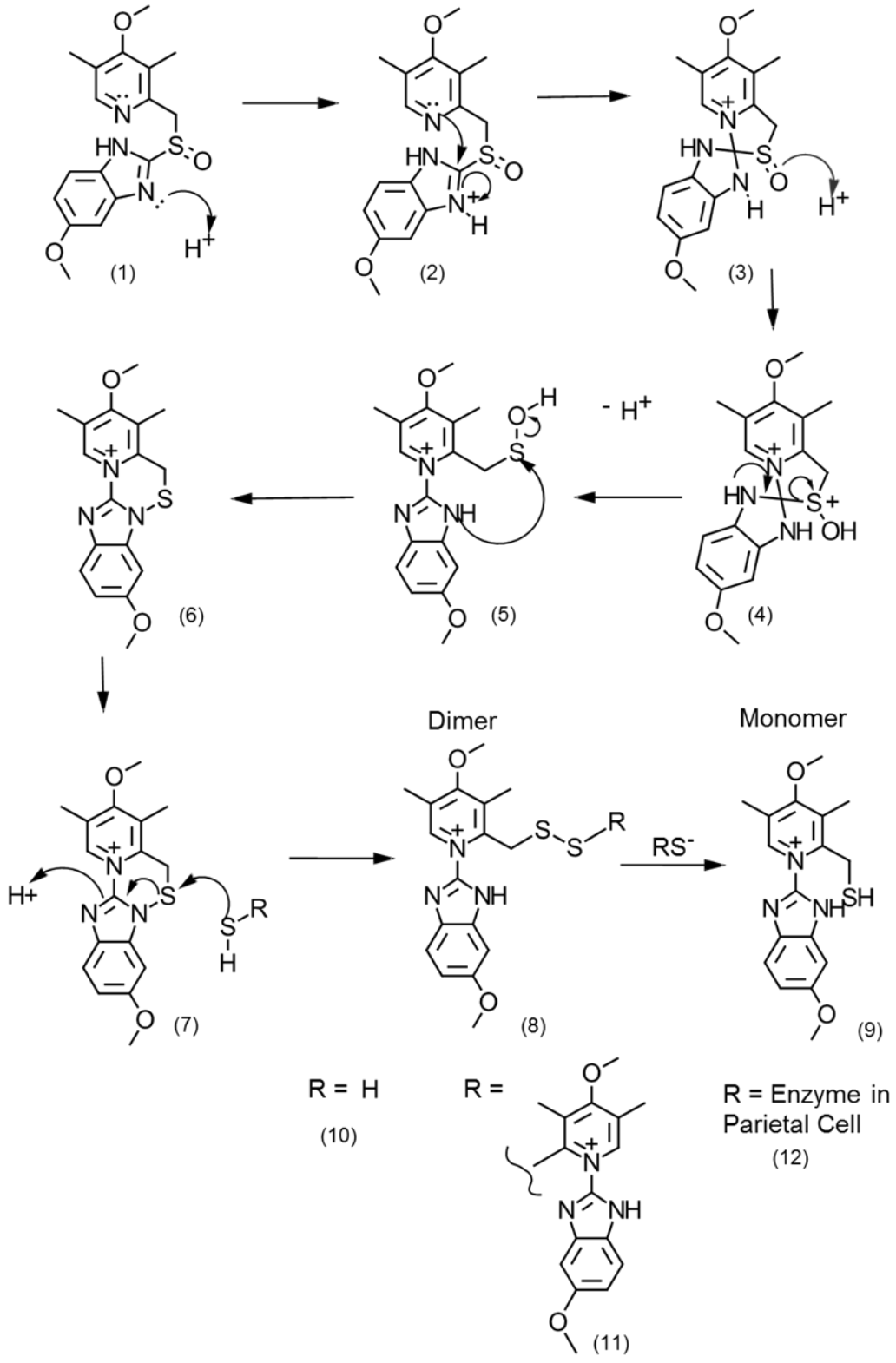


Figure 3. Product ion spectra of the degradant and omeprazole which co-elute. The top spectrum is the dimer degradant with the proposed structure and the bottom is the spectrum for omeprazole. Potential interference could occur between the A+2 isotope of the degradant and omeprazole if data is poorly interpreted.



Scheme 1. Proposed mechanism for monomer and dimer formation on acid treatment of omeprazole (H-S-R will be present in the acidic solution) and the enzyme block in the parietal cell (12). Based on the acid re-arrangement and mechanism of action for the drug on the target enzyme[3,23–26,33].