ADVANCED OXIDATION TECHNOLOGIES FOR THE DECOMPOSITION OF SELECTED ACTIVE PHARMACEUTICALS

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ABSTRACT

Effect of Advanced Oxidation Processes (AOPs) such as H_2O_2/UV, Fenton, photo-Fenton (F-F) and microwave Fenton (MW-F) on pharmaceuticals decomposition have been investigated. The tested compounds were acyclovir (ACY), cyclobenzaprine (CBP), lamotrigine (LAM) and metoclopramide (METO). Factors affecting the process efficiency, i.e.: initial concentration of API (active pharmaceutical ingredient), H_2O_2 and $FeSO_4 \cdot 7H_2O$ (Fe), the impact of UV or MW radiation and presence of four inorganic salts (NaCl, Na_2SO_4, $NaNO_3$ or Na_2CO_3,) were evaluated. The APIs decay increase with increasing hydrogen peroxide excess in H_2O_2/UV system. Photo-Fenton reaction has been proven to be highly efficient in METO decomposition. At higher concentration of each API, decrease in the rate of oxidation was observed. Photocatalytic degradation of METO was significantly limited in the presence of chloride anions. The presence of carbonate ions retarded oxidation of LAMO and ACY in Fenton reaction. HPLC-MS techniques were successfully applied to the analysis of products formed as a result of degradation processes.

Keywords: Advanced Oxidation Processes, acyclovir, cyclobenzaprine, lamotrigine, metoclopramide.

1. Introduction

The meticulous monitoring of pharmaceuticals and personal care products (PPCPs) in the environment has recently gained much attention due to their possible negative impact on human health and on the environment. The presence of small concentration of PPCP has been associated with chronic toxicity, endocrine disruption that includes feminization of male fish and the development of pathogen resistance. The consequences are particularly worrying for aquatic organisms as they are subjected to multigenerational exposure [Sheahan *et al*., 2002, Bouissou-Schurtz *et al*. 2014]. To date, several active compounds have been found in influent and effluent of wastewater treatment, surface water, underground water as well as in tap water in different regions of world [Stackelberg *et al*., 2007; Yu and Chu, 2009; Romero *et al*., 2014]. Most of these compounds enetrates into the environment due to ineffective work of conventional activated sludge wastewater treatment. Therefore effective, highly destructive wastewater treatment technologies are needed. One possible approach is the use of Advanced Oxidation Processes (AOPs), which provide almost complete mineralization of many organic pollutants. It also allows increasing their biodegradability [González *et al*., 2007; Badawy *et al*., 2009]. Among the advanced oxidation methods, H_2O_2/UV method provides decomposition of ketoprofen, prednisolone, pindolol [Wols *et al*., 2013] and clofibric acid [Giri *et al*., 2011]. Fenton reaction affords a complete degradation of sulfametrazine [Pérez-Moya *et al*., 2010], acetaminophen, bisophenol-A [Li. *et al*., 2012] and diclofenac [Basavaraju, 2012]. Photo-Fenton is sufficient to decompose amoxicillin [Elmolla and Chaundhuri, 2009], paracetamol [Basavaraju, 2011] and gabapentin [De la Cruz *et al*., 2012]. The success of those methods is justified by reactions of organic compounds with very reactive oxidant (hydroxyl radicals ($HO^{\cdot}$): (E° = 2.8 V vs. standard hydrogen electrode (SHE)).

In the present work, the applicability of various Advanced Oxidation Processes for the degradation of acyclovir, cyclobenzaprine, lamotrigine and metoclopramide were tested under laboratory conditions. The influence of parameters such as H_2O_2 and Fe dosages, UV illumination, MW radiation or presence of inorganic salts were considered. New intermediate degradation products have been described.

2. Materials and methods

Acyclovir (ACT), cyclobenzaprine hydrochloride (CBP), lamotrigine (LAMO) and metoclopramide (METO) were donated by local pharmaceutical company. Ferrous sulfate heptahydrate (99%), methanol and acetonitrile (HPLC-grade) were purchased from Sigma Aldrich. Hydrogen peroxide (30%), potassium dihydrogen phosphate, sodium hydroxide, sulfuric acid, sodium chloride, sodium nitrate, sodium bicarbonate were purchased from P.P.H. Gliwice (Poland). All standards including API solutions were prepared in deionized water. The procedure of API degradation was exactly the same as in (Kamińska *et al*., 2015). All experiments were done in triplicate to obtain the mean value. The relative standard deviation is not reported on the graphs for the sake of clarity; nevertheless, it was below ±10% in all analyses. HPLC-DAD-MS/MS analyses were carried out using RP-HPLC (Agilent 1200 Series). Analyses were performed on Zorbax SB C18 column (150 mm x 4.6 mm, 5 µm (Agilent, USA)). For ACY, the mobile phase was the same as given (Chaudhari and Ubale, 2012) and used under isocratic conditions. The detector was set at wavelength 264 nm. CBP was analysed using 0.1% HCOOH (v/v) as eluent A and acetonitrile as eluent B. CBP was analysed with the following gradient elution conditions (min/A%): 0/45, 5/60, 6/45, 10/45. DAD set at 224 nm. LAMO was analysed using 0.025 M $HCOONH_4$ as eluent A and 0.1% TFA in acetonitrile as eluent B. LAMO was separated with the following gradient elution conditions (min/A%): 0/20, 10/50, 11/20, 15/20. DAD set at 210 nm. For METO a mobile phase containing potassium dihydrogen phosphate (pH 3) and acetonitrile (60:40) used under isocratic mode and samples were monitored at 275 nm. The UHPLC-QTOF-MS analysis were carried out using an Agilent 1290 UHPLC system coupled to a hybrid quadrupole time-of-flight (QTOF) mass spectrometer (Agilent 6540 Series Accurate Mass QTOFMS) with Dual ESI interface operated in positive ion mode. Briefly, the separation of analytes was performed on Lichrocart 250-4 Lichrospher 100 RP-18e column (250 mm x 4 mm; 5µm) maintained at 40 °C. The mobile phase A was ultrapure water and B was acetonitrile and the flow rate was set to 1 ml/min. The analysis were carried out in gradient elution (starting from 80% A to 20% A in 15 min, back to 80% A in 6 min and kept at 80% A for 9 min) and the injection volume was 5µL. The QTOF-MS conditions were as follows: sheath gas temperature 400 °C at the flow rate of 12 L/min, capillary voltage 3500 V, nebulizer pressure 35 psig, drying gas 10 L/min, gas temperature 300 °C, skimmer voltage 45 V, octopole RF peak 750 V and fragment or voltage 100 V. Analysis were performed in two different modes, MS/MS or Target MS/MS with various collision energies (10, 20 and 30 V) and the masses were scanned from 100 to 1000 m/z. The instrument was working in the 4 GHz high-resolution mode with the acquisition rate of 1.5 spectra/s. Acquisition data were processed using Agilent Mass Hunter Workstation software.

3. Results and discussion

Four pharmaceuticals of different structures and chemical reactivity were used as reagents in Advanced Oxidation Processes (Figure 1).

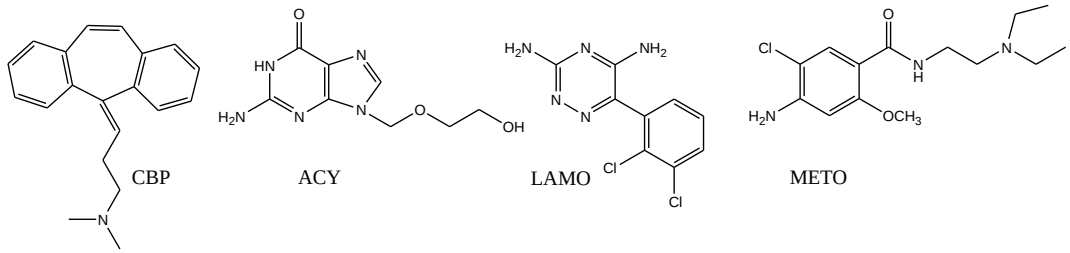
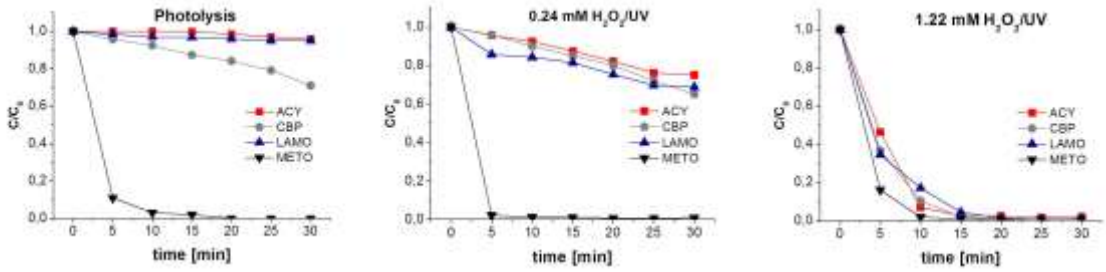


Figure 1: Chemical structures of used APIs.

The tested APIs are resistant to H_2O_2.The oxidation reaction takes place in less than 5% (0.24 mM to 4.88 mM H_2O_2). The direct photolysis is sufficient only for METO; its total decomposition is observed after 20 min (Figure 2). In the UV/H_2O_2 system, at an initial concentration of 0.1 mM for ACY and LAMO (irradiation time 15 min) with the H_2O_2 dosage from 0.24 mM to 4.88 mM, the ACY and LAMO removal were increased from 13% to 95.5% (ACY) and from 19% to 99.4% (LAMO), indicating that UV/H_2O_2 method was superior over UV alone in terms of the removal efficiency. For CBP and METO, the optimum H_2O_2 dosage was 2.44 mM and 1.22 mM, respectively. Higher H_2O_2 concentration retarded their decomposition rate. It can be caused by quenching effect of hydroxyl radicals (Aleboyeh *et al*., 2005). Overdose of oxidation agent indicate generation of hydroperoxyl radical ($HOO^{\bullet}$) [Christensen *et al*., 1982] less reactive than $HO^{\bullet}$ and able to further consume valuable hydroxyl radicals. The best result of Fenton rection with small amout of oxidant (0.24 mM H_2O_2 and 0.09 mM Fe) proceeds only with CBP, which was fully removed after 20 minutes. The remaining tested APIs were eliminating inefficiently. Much better results were obtained in photo-Fenton reaction, which provided the overall decomposition of METO, CBP and LAMO after 5, 20 and 50 minutes, respectively. The effectiveness of Fenton reaction could be also improved using larger amount of oxidant. For example, CBP was completely removed after 1 min if 1.22 mM H_2O_2 and 0.18 mM Fe, and LAMO was degraded within 30 min when correspondingly 2.44 mM H_2O_2 and 0.18 mM Fe were used. ACY was quite resistant towards Fenton reaction. Its entire decomposition was not achieved even after 30 min of 4.88 mM H_2O_2 and 0.72 mM Fe application. Nevertheless, ACY could be fully destroyed by photo-Fenton reaction (1.22 mM H_2O_2 and 0.18 mM Fe). In each case, photo-Fenton process provides higher mineralization than Fenton assisted with MW radiation (Figure 2). Note the fact that increase the initial APIs concentration decreases efficiently the decomposition rate.

The effect of four different inorganic anions commonly occurring in natural water (chloride, nitrate, sulfate and carbonate) on APIs degradation was tested. In each experiment, salts were added to the solution 0.1 mM (200 mL of particular API) as a solid in an amount to provide 10 mM concentration. The experiments were carried out under the previously selected conditions (1.22 mM H_2O_2 in H_2O_2/UV system, 1.22 mM H_2O_2 /0.18 mM Fe in Fenton reaction and 0.24 mM H_2O_2 /0.09 mM Fe in photo-Fenton reaction). In the case of H_2O_2/UV reaction, the addition of salts revealed inhibiting effect on METO degradation that follows the order: $NaNO_3$ < NaCl < Na_2SO_4 < Na_2CO_3. Sodium nitrate retarded CBP degradation in 40%, sodium sulfate slowed down ACY decomposition by 14%. The most important role in the degradation of API by H_2O_2/UV reaction played hydroxyl radicals formed by photolysis of hydrogen peroxide. It has been reported that not only H_2O_2/UV but also Fenton reaction efficiency can be poorer in the presence of salts, due to the formation of less reactive complexes ion-Fe(II). Moreover, new radicals (e.g. $Cl^{\bullet-}$, $Cl_2^{\bullet-}$) are formed that also are less reactive than $HO^{\bullet}$. Nitrate ions slowed down CBP degradation. Chloride ions caused deleterious effect on LAMO decomposition. The degradation rate of METO was reduced approximately by 24%, 29%, 40% and 95% in the presence of NO_3^-, SO_4^{2-}, Cl^-, CO_3^{2-}, respectively. Effect of inorganic ions on APIs photo-Fenton degradation is negligible.



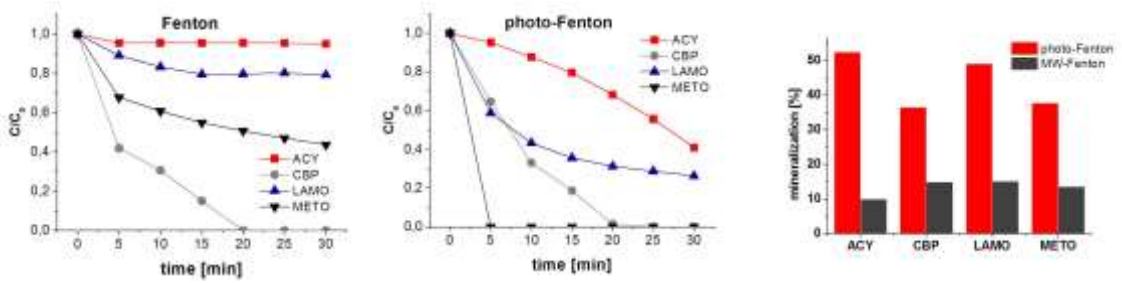


Figure 2: The influence of various AOPs on the APIs degradation. Fenton and photo-Fenton reaction API [0.1 mM], Fe [0.09 mM], H_2O_2 [0.24 mM].

In order to establish what are the oxidation products formed initially, HPLC-MS/MS analyses of the partially degraded APIs solution (F-F, 10 min.) were performed. Excess of H_2O_2 was eliminated by freeze-drying the solution and then diluted it 1/100 v/v prior to injection into the HPLC-MS system. The chromatograms showed many peaks, and corresponding chemical structures are proposed on the basis of a series of successive MS/MS analyses. The successive fragmentations in the ion trap evinces losses of uncharged species (CO, CO_2, H_2O, HCOOH, NH_3, HCl etc.) compatible with the chemical structures proposed for the most important chromatographic peaks.

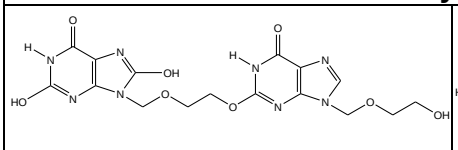
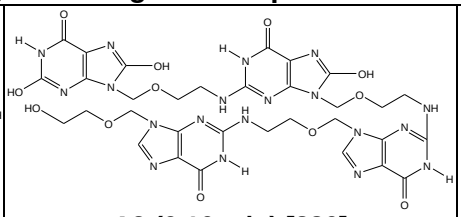
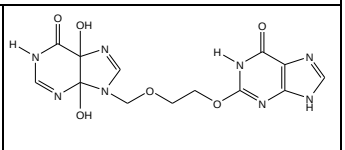
Used in the tests compounds are amines, possessing differ chemical reactivity. Acyclovir (2-amino-1,9-dihydro-9-((2-hydroxyethoxy)methyl)-6*H*-purin-6-one) is reasonably resistant towards generated hydroxyl radicals in AOP reactions. The observed degradation products are derivatives having hydroxyl group(s) in 2, 4, 5 or 8 positions. The primary route of acyclovir decomposition is dimerization and trimerization of radical forms.

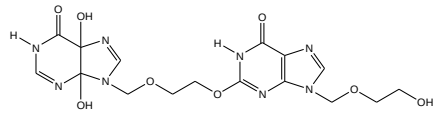
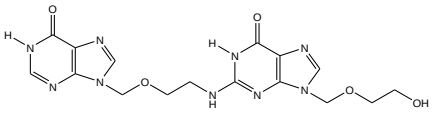
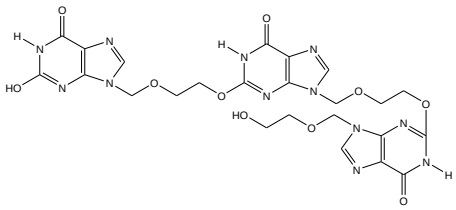
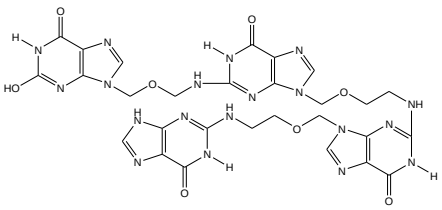
Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine) due to the presence of two amino groups and two chlorine atoms is good substrate in radical reactions. Unfortunately, a large group of degradation products are compounds with a more complex structure. Post-reaction mixtures (MW-F and F-F) contained products of: triazole ring opening, the elimination of chlorine atoms and/or amino groups, and substitution of the benzene ring.

Cyclobenzaprine (3-(5*H*-dibenzo[a,d]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine) is easily oxidizable. The first step is addition of the hydroxyl radical to cycloheptene double bond. As a result of next oxidation step the C-C bond is broken. Post-reaction mixtures (MW-F and F-F) contained many carbonyl compounds and alcohols.

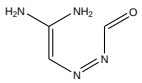
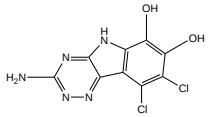
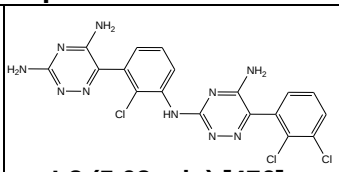
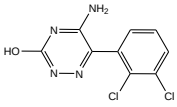
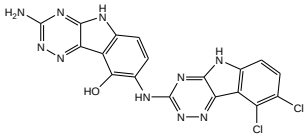
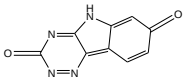
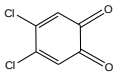
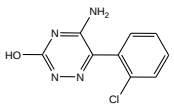
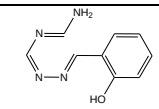
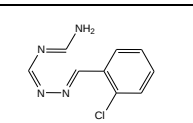
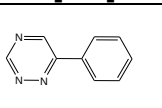
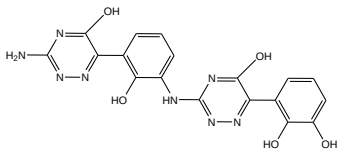
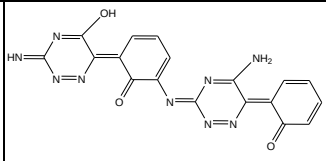
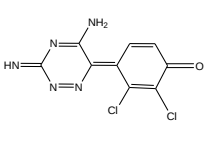
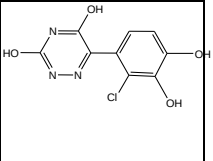
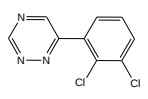
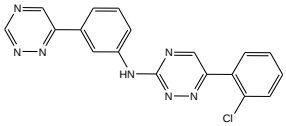
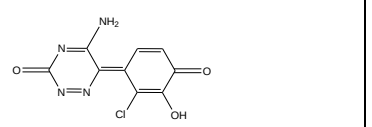
Metocroplamide molecule (4-amino-5-chloro-*N*-(2-(diethylamino)ethyl)- 2-methoxybenzamide) contains two substituents particularly reactive in radical reactions (chlorine, and amino group). The formed radicals initiate the oligomerization reactions. Additionally, substitution of the amino groups and hydroxyl radicals chlorine atom takes place.

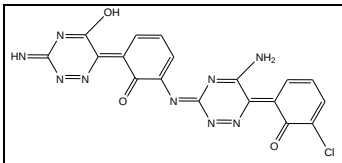
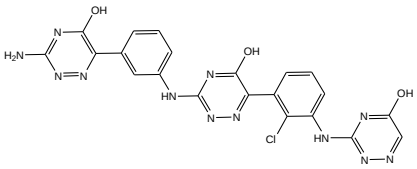
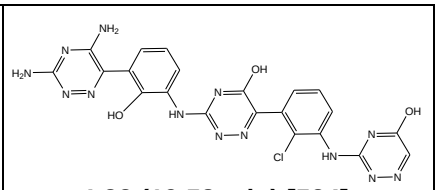
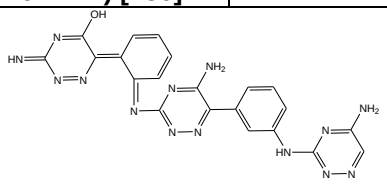
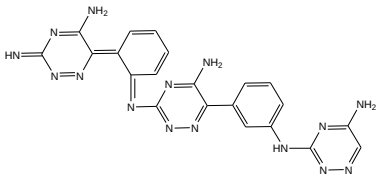
Table 1: *m*/*z* values and retention time of major photo-Fenton products of ACY, LAMO, CBP and METO.

Structure of M, (retention time) and m/z [M + H]		
Acyclovir degradation products		
		
A1 (2.30 min) [451]	**A3 (6.10 min) [880]**	**A5 (13.90 min) [379]**

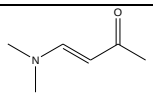
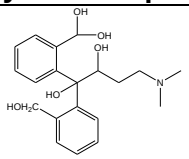
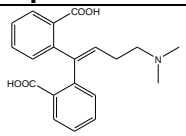
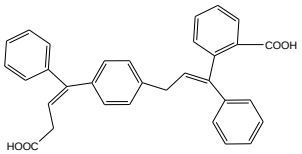
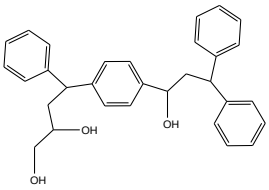
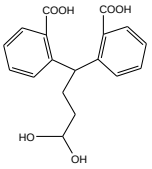
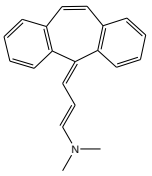
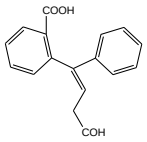
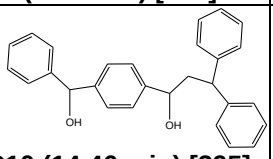
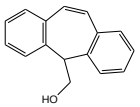
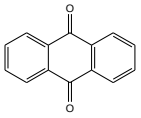
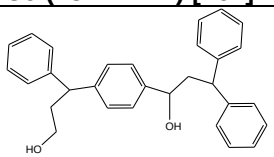
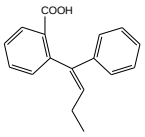
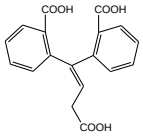
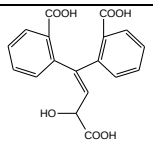
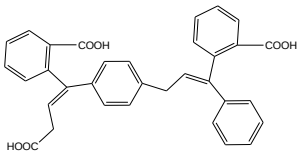
	
A2 (5.84 min) [453]	**A4 (13.62 min) [418]**
	
A6 (14.99 min) [643]	**A7 (21.58min) [744]**

Lamotrigine degradation products

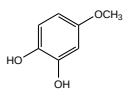
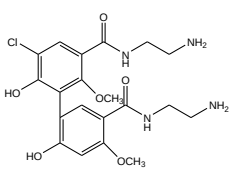
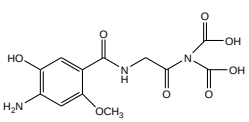
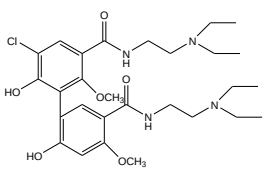
			
L1 (3.40 min) [115]	**L2 (3.66 min) [287]**	**L3 (5.03 min) [476]**	**L4 (6.60 min) [258]**
			
L5 (8.84 min) [454]	**L6 (12.77 min) [129]**	**L7 (13.12 min) [201]**	**L8 (14.71 min) [177]**
			
L9 (14.72 min) [223]	**L10 (14.89 min) [191]**	**L11 (15.05 min) [209]**	**L12 (15.23 min) [158]**
			
L13 (16.15 min) [423]	**L14 (16.21 min) [402]**	**L15 (16.60 min) [256]**	**L16 (16.77 min) [227]**
			
L17 (16.99 min) [362]	**L18 (17.00 min) [253]**	**L19 (17.12 min) [239]**	

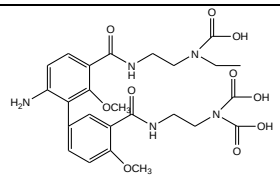
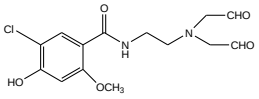
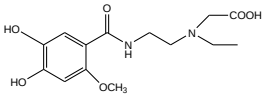
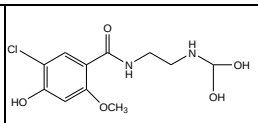
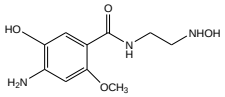
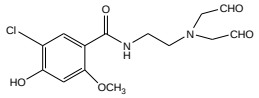
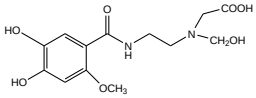
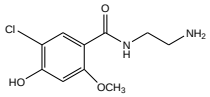
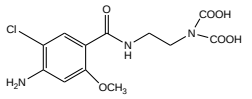
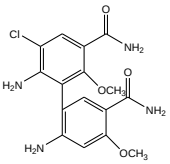
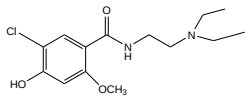
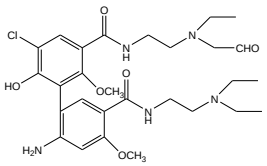
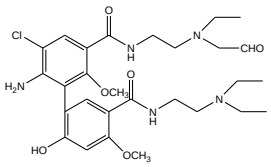
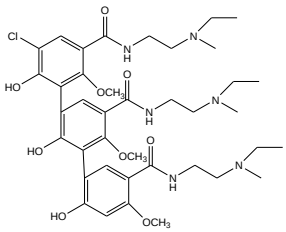
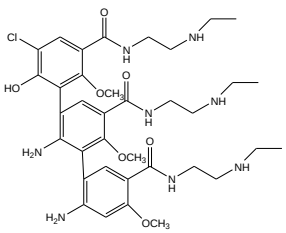
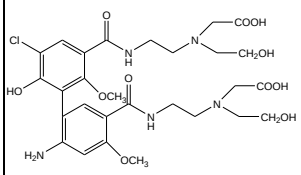
 L20 (17.67 min) [436]	 L21 (18.38 min) [519]	 L22 (19.58 min) [534]
L23 (22.73 min) [481] 	 L24 (22.77 min) [480]	

Cyclobenzapine degradation products

 C1 (3.14 min) [114]	 C2 (5.12 min) [362]	 C3 (6.56 min) [340]	 C6 (12.33 min) [129]
 C4 (8.36 min) [475]	 C4 (8.36 min) [475]	 C7 (12.36 min) [331]	 C8 (13.15 min) [274]
 C9 (13.71 min) [267]	 C10 (14.46 min) [395]	 C11 (14.64 min) [223]	 C12 (14.98 min) [209]
 C13 (15.99 min) [423]	 C14 (16.98 min) [253]	 C15 (17.66 min) [327]	 C16 (17.98 min) [343]
 C17 (18.39 min) [519]			

Metocroplamide degradation products

 M1 (1.94min) [141]	 M2 (7.38 min) [453]	 M3 (8.99 min) [356]	 M4 (9.36 min) [566]

 **M5 (9.36 min) [604]**	 **M6 (10.54 min) [329]**	 **M7 (10.55 min) [313]**	 **M8 (10.55 min) [291]**
 **M9 (10.62 min) [342]**	 **M10 (10.87 min) [329]**	 **M11 (12.68 min) [315]**	 **M12 (14.42 min) [245]**
 **M13 (15.24 min) [332]**	 **M14 (16.23 min) [365]**	 **M15 (18.51 min) [301]**	 **M16 (18.52 min) [579]**
 **M17 (18.75 min) [579]**	 **M18 (23.30 min) [788]**	 **M19 (23.67 min) [744]**	 **M20 (23.85 min) [644]**

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