Analysis of metabolites from beta blocking drugs in biological samples using an Agilent 1100 Series capillary LC/Trap XCT system

Application

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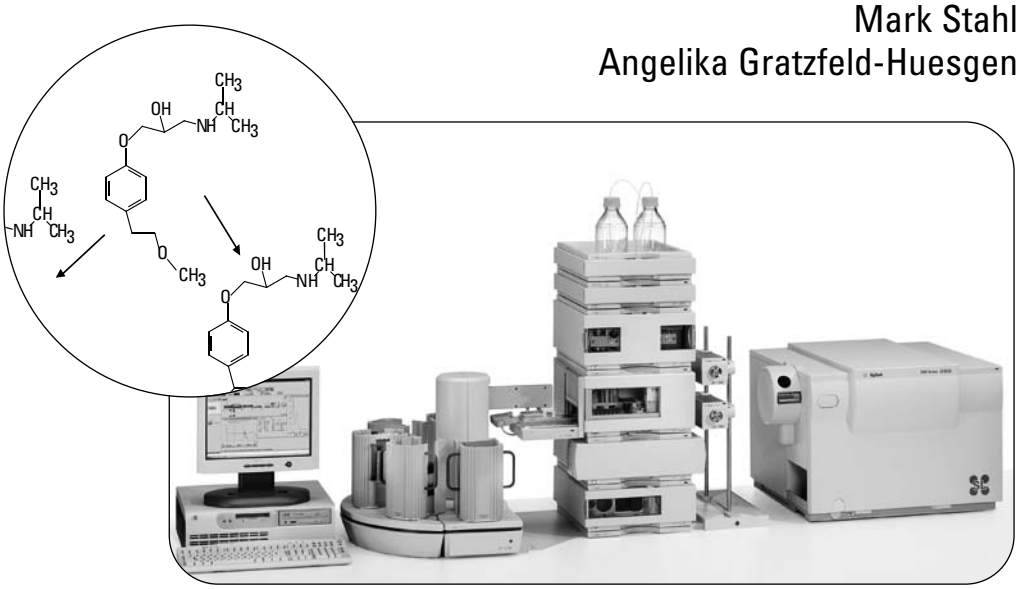

Abstract

This Application Note describes how a S9 human liver homogenate was used to simulate oxidative phase 1 metabolism of the beta-blocking drugs propranolol, metoprolol and pindolol. Afterwards, the samples were partially purified by precipitation/extraction with trifluoroacetic acid and hexane, followed by analysis with an Agilent 1100 Series LC/MSD trap XCT system. Fully automated LC/MS and LC/MS^n analysis allowed for identification of the metabolites produced by the liver homogenate.

Agilent 1200 Series Verified

This application has been verified using an Agilent 1200 Series LC system, and showed comparable or even better performance.

Application Area
Identification of metabolites in drug development

Agilent Technologies

Introduction

The analysis of drugs and their metabolites in biological samples is a difficult application common in the fields of toxicology, forensics and pharmacokinetics (ADME). This application has increased in importance over the last few years due to the rise of metabolomics research. Previously, many of these analyses were done by GC/MS. Today, LC/MS is favored because it allows analysis of drugs and metabolites of higher molecular weight and also permits analysis of thermally labile compounds. Furthermore, derivatization of the drugs or metabolites is usually not required for LC/MS analysis, and this generally leads to better detection limits and shorter overall analysis times. For detection, all MS types are useful, and each type has its advantages and disadvantages. A quadrupole instrument offers high sensitivity and good quantitative accuracy in SIM mode. A time of flight (TOF) mass spectrometer provides the user with highly accurate masses, thus allowing the determination of the most probable molecular formula in combination with high sensitivity. An ion trap instrument allows highly sensitive MS^n measurements. In addition to accurate quantitation, the ion trap MS^n operation allows structural characterization of drug metabolites, many of which are unknown and present in minute amounts only.

Beta-blocking drugs are widely used for the treatment of cardiovascular diseases and are also beneficial in the treatment of hypertension, hyperthyroidism, tremor, migraine or glaucoma. A great number of adrenoreceptor blocking drugs are available which differ widely in pharmacodynamic and pharmacokinetic parameters. In this Application Note, we demonstrate the usefulness of a capillary HPLC-ion trap system for the identification of several beta-blocking drug metabolites produced under simulated in vivo conditions.

Experimental

Simulation of metabolism

Oxidative phase 1 metabolism of the beta-blockers propranolol, metoprolol and pindolol was simulated using an S9 human liver homogenate often used in toxicological studies such as the Ames test. It includes the main enzymes responsible for the metabolism of xenobiotics, for example, the Cyt P_{450} family. Several enzymes have not been included in the assay, as only phase 1 metabolites of the beta-blockers are formed, because the S9 human liver homogenate does not contain all enzymes involved in metabolism and substrates/cofactors. Thus, only a subset of the important metabolic pathways are addressed with this approach, however, it nevertheless provides useful information about the metabolism of these drugs.The beta-blocking drugs propranolol hydrochloride, metoprolol tartrate and pindolol (all Sigma, Taufkirchen, Germany) were used. A solution of the human S9 liver homogenate (Molecular Toxicology Inc., Boone, USA) was prepared under sterile conditions according to the manufacturer's directions. This included the addition of glucose-6-phosphate, NADPH, KCl, $MgCl_2$ and adjustment of the pH to 7.4 using sodium phosphate buffer. An aliquot of 100 µL of an aqueous solution of one of the beta-blocking drugs was added to 900 µL of the liver homogenate preparation so that the final assay concentration was 1 µg/mL. The assay was incubated at 37 °C for 35 hours.

Sample preparation

An aliquot of 400-µL sample was spiked with 400-µL hexane and 20 µL trifluoracetic acid. After mixing thoroughly, the sample was centrifuged (12000 g, 15 min, 4 °C). The organic phase and boundary layer were removed, and the aqueous phase was transferred to a new sample vial. The precipitation/extraction procedure was repeated, and the resulting aqueous fraction was analyzed.

Sample analysis

The prepared sample (0.5 µL) was analyzed with an Agilent 1100 Series LC/MSD Trap XCT system. The key acquisition parameters are listed in table 1.

Chromatographic conditions:
Agilent 1100 Series capillary LC system

Column:	ZORBAX SB C18, 0.3 x 150 mm, 3.5 µm
Mobile phase:	A = water (0.1 % formic acid)
	B = acetonitril (0.1 % formic acid)
Flow rate:	4 µL/min
Gradient:	0 – 5 min: 3% B
	5 – 65 min 95 % B
	65 – 80 min 95 % B
Post time:	20 min

Trap XCT MS conditions:
Agilent XCT ion trap mass spectrometer

Capillary voltage:	4000 V	Automatic MS/MS:	
Drying gas flow:	4 L/min	Peptide scan mode	
Drying gas temp:	320 ºC	Number of parents:	4
MS Scan range:	150 – 1500 Da	Averages:	2
Averages: 3		Fragmentation:	1.3 V with SmartFrag
ICC: Maximum accumulation time: 50 ms			30 – 200 %
Smart Target: 120000		Active Exclusion:	2 spectra, 1.5 min

Table 1
Analytical conditions

Results and discussion

The TIC chromatogram and representative spectra from the analysis of simulated metabolism of metoprolol is shown in figure 1. The chromatogram is quite clean with little evidence of lipids and proteins.

Chromatographic retention time, mass spectra and MS/MS spectra of metoprolol allow identification of three metabolites: α-hydroxy-metoprolol, o-desmethyl-metoprolol and a further metabolized carbonic acid derivative, which is the main metabolite of metoprolol[1]. Propranolol and pindolol also become hydroxylated under digestion conditions. Due to the limited enzyme complement of the S9 liver homogenate, and the lack of substrates/cofactors only oxidative phase one metabolites were formed (figures 1, 2 and 3). For a more complete distribution of metabolism products to be obtained, other phase 1 and phase 2 enzymes and substrates have to be added. All the metabolites found agree with those in reference literature[1-4].

This work demonstrates that the Agilent 1100 Series LC/MSD trap XCT system is useful for detecting and structurally characterizing drug metabolites. The results clearly demonstrate that this sample preparation method[5] is capable of extracting phase 1 metabolites of beta-blocking drugs from a crude cellular matrix and removing lipids and proteins present in high concentrations in the sample. Although no quantification was done in these experiments, a rough quantification based on the drug's calibration curve or a more sophisticated quantification based on individual calibration curves for the single metabolites is possible.

Conclusion

This Application Note demonstrates that a S9 human liver homogenate can be used to simulate the oxidative phase 1 cytochrome P450-based metabolism of drugs. For sample preparation, a combination of acid and organic solvent precipitation/extraction was used to remove proteins and lipids. The different metabolites of the beta-blocking drugs were identified and structurally characterized with an Agilent 1100 Series LC/MSD trap XCT system, which permitted fully automated analysis of these compounds at very low concentrations.

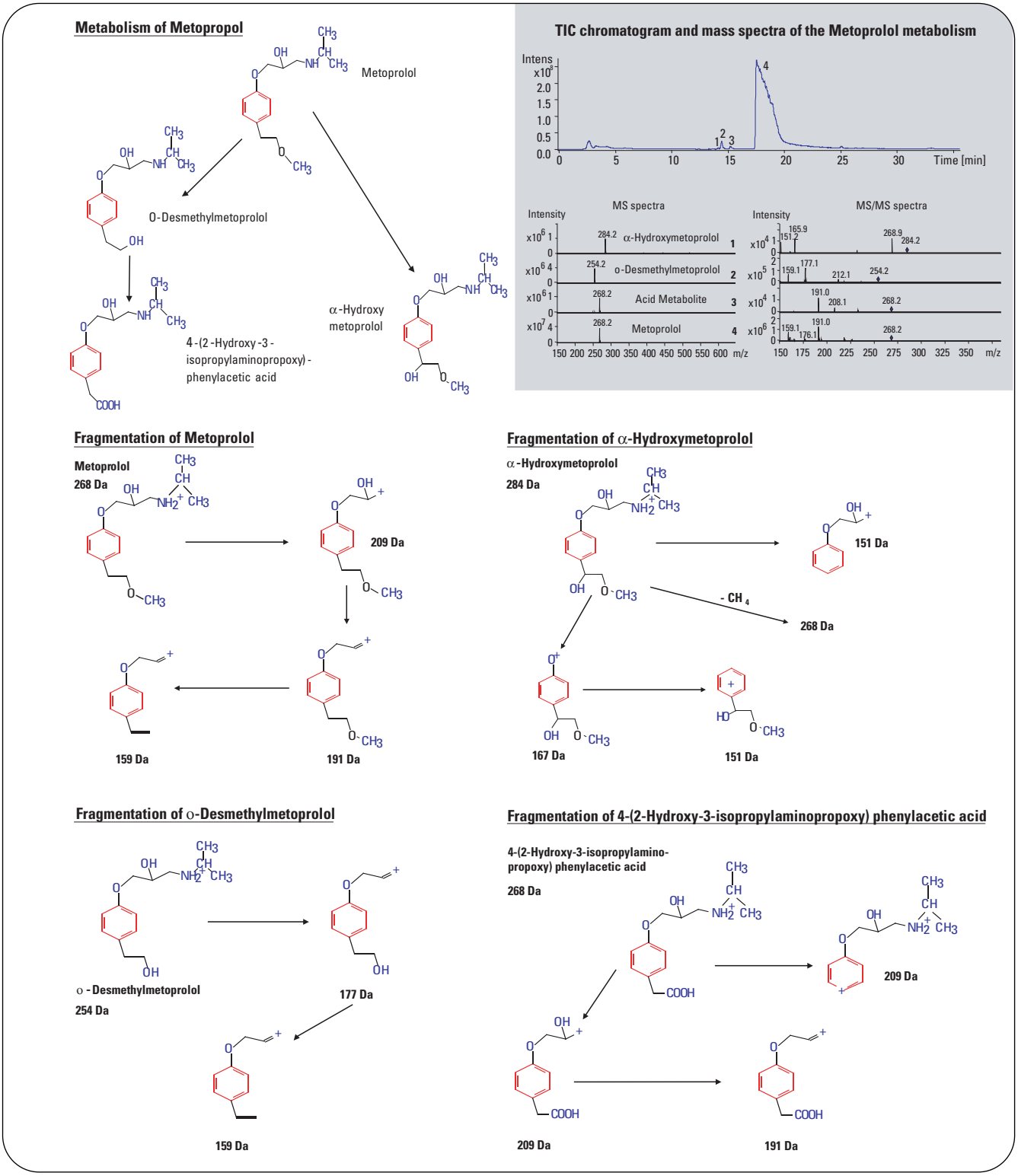

Figure 1
Cyt P_{450} dependent metabolism of metoprolol. Total ion chromatogramm, MS and MS/MS spectra of the analysis and corresponding fragment ions.

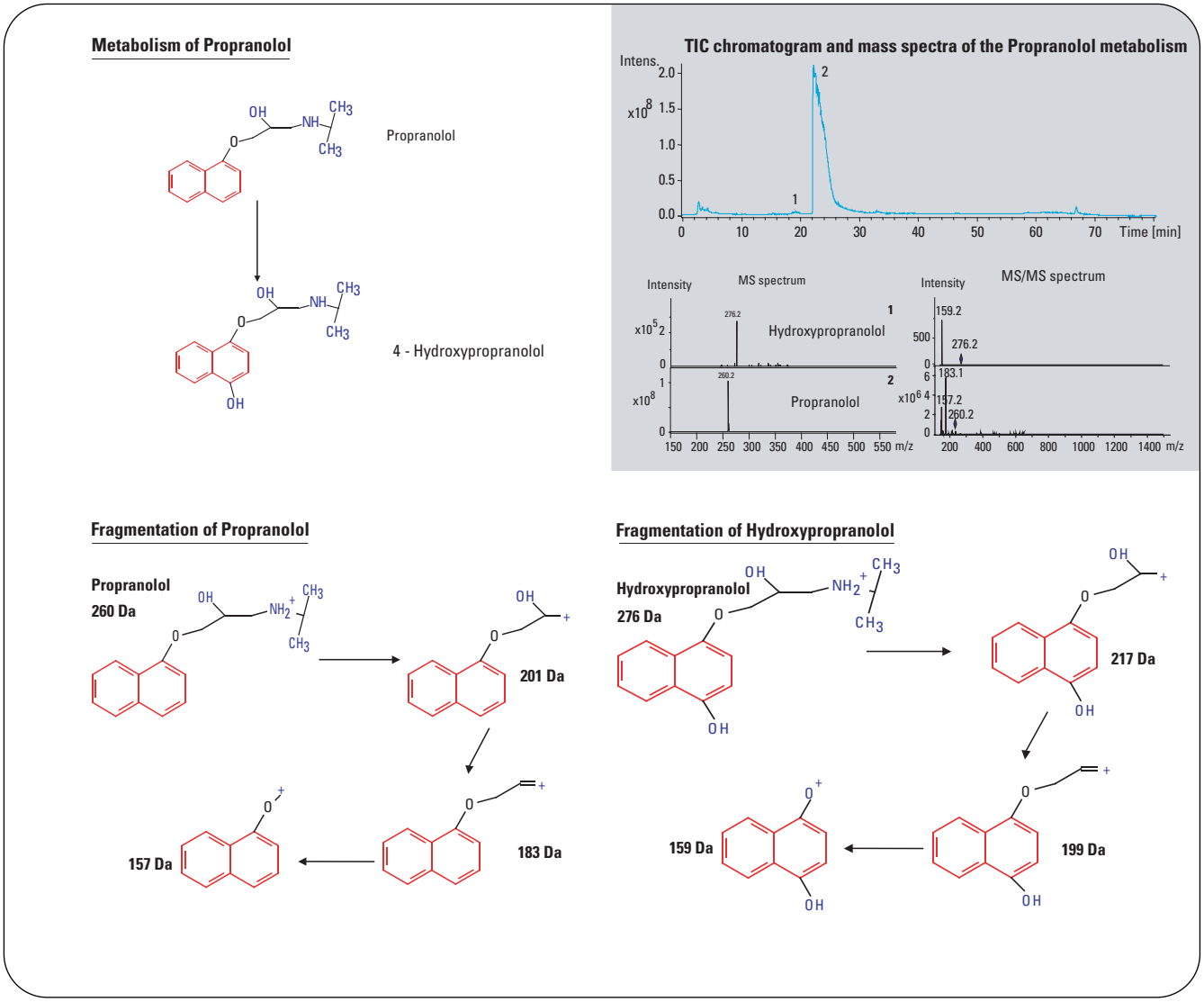

Figure 2
Cyt P_{450} dependent metabolism of propranolol. Total ion chromatogramm, MS and MS/MS spectra of the analysis and corresponding fragment ions.

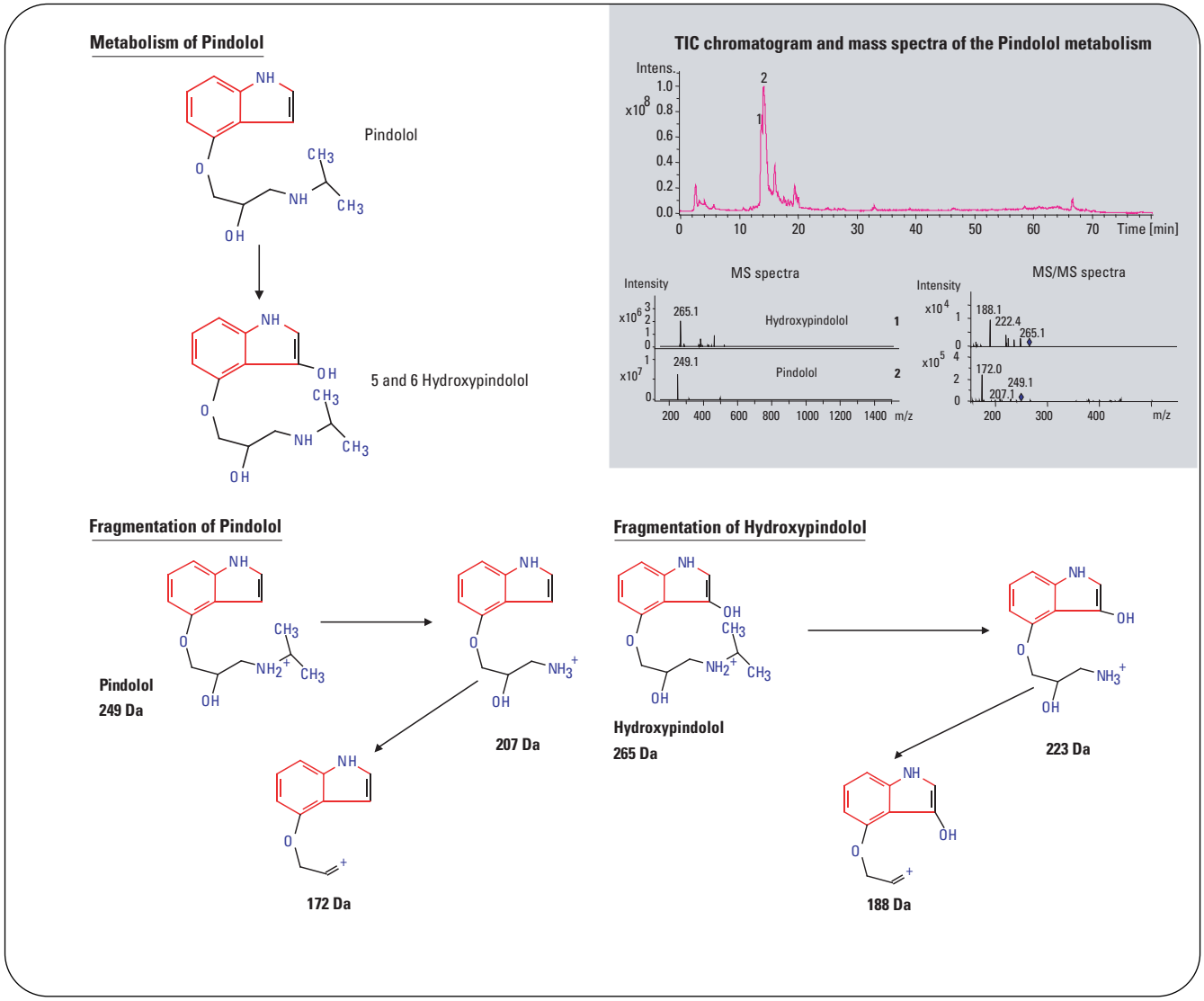

Figure 3
Cyt P_{450} dependent metabolism of pindolol. Total ion chromatogramm, MS and MS/MS spectra of the analysis and corresponding fragment ions.

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