

Identification of Metabolites of Haloperidol in Rat Liver Microsomes Using Microflow LC/MS/MS

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Abstract:

When coupled to a triple quadrupole mass spectrometer, microflow HPLC offers significant advantages in analysis speed and chromatographic resolution for the identification of drug metabolites in biological media. We investigate systematically the detection and identification of metabolites of haloperidol in rat liver microsomal preparations using a reverse phase microflow HPLC gradient elution method with the mass spec operating in the multiple reaction monitoring mode. The chromatographic resolution and quality of the mass spec data are evaluated with respect to the number of identifiable and quantifiable metabolites. The value of using rapid gradient microflow LC/MS/MS for a quick profiling and semi-quantitative estimate of metabolites from candidate compounds in early drug discovery is discussed.

Experimental:

Microflow HPLC System:

A single-channel capillary HPLC system from Eksigent Technologies with the integrated CTC/PAL autosampler was used for the LC/MS experiments. The microflow system is optimized for column ID of 0.3 mm and flow rates in the 1 - 30 μ L/min range.



Mass Spectrometry:

An API 3200 Q Trap from Applied Biosystems equipped with a TurboV ion-spray source was employed for the LC/MS/MS studies. A short-length (< 50 cm) of 125/30 μ OD/ID fused silica transfer capillary was used between the UV flow cell and the mass spectrometer. The fused-silica capillary was threaded through the 175 μ ID stainless steel electrospray needle all the way to just past the opening.

Separation Conditions:

Column: Eksigent ChromXP C18 EP, 3 μ , 120 \AA , 50 x 0.3 mm
 Mobile Phase: A: water and B: acetonitrile, both with 0.1% formic acid
 Gradient:

Min	B
0	2 %
1	30 %
9	45 %
9.1	95 %
10	95 %

Temperature: ambient
 Flow Rate: 8 μ L/min
 Detection: UV@215 and 254 nm
 Injection Volume: 1 μ L

Sample Preparation:

100 μ M of Haloperidol was incubated with rat liver microsomes (1 mg/mL) and cofactors for 1 hour. The metabolites were extracted by the ACN protein precipitation method and re-constituted in 90/10 water/ACN solution.

Metabolite ID Workflow

The parent compound Haloperidol was first characterized by MS and MS². The information thus obtained was then used to set up neutral loss, precursor scan, product ion scan, MRM, MS² and MS³ experiments for the detection and identification of metabolites. Analyst[™] Metabolite ID 1.4 was used to facilitate the mass spec data interpretations.

Results:

Microflow LC/MS Method Development

A suitable HPLC method for Met ID studies needs to balance between resolution and speed. In general, the metabolites tend to be more polar and faster eluting than the parent compound while some adducts tend to be late eluting. Many metabolites have the same mass and closely-related structures, for example, mono-hydroxylated metabolites (parent +16) differing only in the position of the hydroxyl group on a hetero cycle ring structure. A general approach is to have a shallow gradient at the beginning to allow the separation of polar metabolites, followed by a steeper gradient to elute the more lipophilic compounds. The last step is a high organic wash period followed by the returning to high aqueous initial condition. A mixture of 4 compounds: acetaminophen, buspirone, verapamil and ketoprofen were used here as a model system for the development of chromatographic separations. Selected results are shown below.

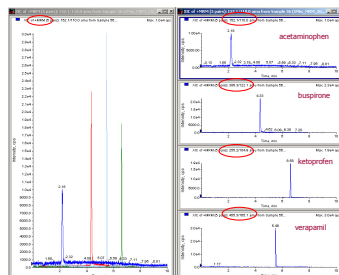


Figure 1 Separation of a mixture of drug compounds representing the typical range of retention times seen in MetID studies. C18 EP, 3 μ , 50 x 0.3 mm. Gradient: 5% B from 0 to 0.5 min; to 25% B, 4 min; to 90% B, 7.5 min; hold at 90% B until 10 min. MRM transitions are circled in red.

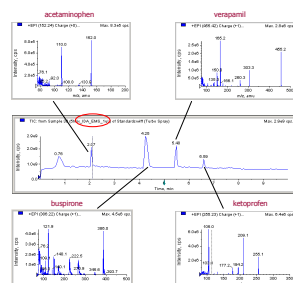


Figure 2 IDA (information dependent acquisition) method with EMS (enhanced mass scan) survey scans and EPI (enhanced production) scans revealed detailed structural information of the compounds from their collision fragment patterns.

Haloperidol Metabolites

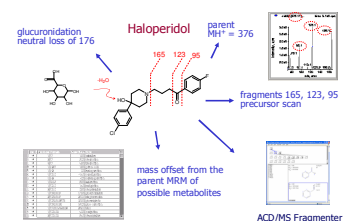


Figure 3 MS/MS characterization of parent compound assisted by MetID software. The information is used for setting up subsequent experiments.

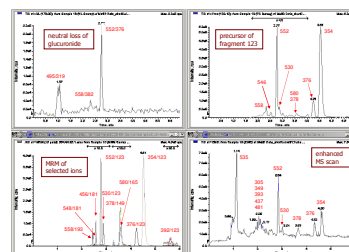


Figure 4 Representative neutral loss, precursor, selected MRM and enhanced scan. Each is a separate injection of extracted haloperidol metabolites. The retention time, mass, type of scan and fragmentation pattern (not shown) from the LC/MS peaks combined with knowledge about the molecular structures provide the basis for the identification of metabolites.

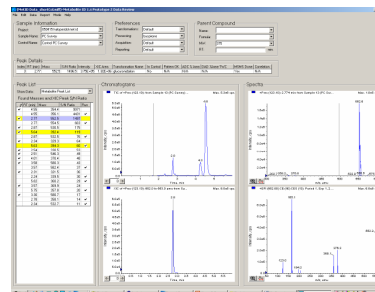


Figure 5 An example of using Analyst MetID software to facilitate the identification of metabolites.

MH+	neutral loss	precursor	MRM	enhanced scan	
354		x	x	x	HPP+
376		x	x	x	HP
378		x	x	x	RHP
392			x	x	HP + (O)
456			x		HP + (SO ₂)
495	x				
530		x	x	x	HPP+ Gluc
546		x			HPP+ Gluc + (O)
548			x		
562	x	x	x	x	HP Gluc
568	x		x		
580			x		
Total	3	8	10	5	

Table 1 Total number of identified metabolites from this study. For mass and structure designations see the biotransformation reaction scheme shown next.

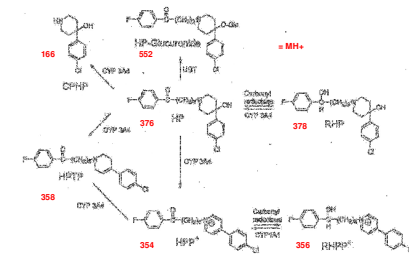


Figure 6 Proposed scheme of biotransformation of Haloperidol

Conclusions:

Metabolite profiling is an important step in the drug discovery process to ensure the safety and efficacy of drug candidates. High resolution HPLC using 4.6 or 2.1 mm ID columns with a typical run time of 20 to 60 minutes are routinely used for LC/MS/MS MetID studies. We demonstrate here that microflow HPLC, which uses 0.3 mm ID column, offers benefits of fast gradient, low delay volume, rapid re-equilibration and improved chromatographic resolution. The haloperidol metabolites identified are in good agreement with available information in the literature. There is an increasing need for faster analysis and better data quality for LC/MS based MetID techniques. Microflow HPLC coupled to a Q Trap mass spec is well suited to serve this purpose.