Screening and Semi-Quantitative Analysis for Pharmaceutical Drug Metabolites in Urine Samples

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MPR400

Introduction

The metabolites of ibuprofen in urine samples derived after administration of a cold medicine were screened and measured using a QQQ and a Q-TOF LC/MS system.

MRM semi-quantitative analysis for ibuprofen metabolites in urinary samples was performed using ketoprofen for internal standard purposes.

Furthermore, non-target metabolites were identified using accurate mass data from a Q-TOF LC/MS and applications software for metabolite identification.

During 0 hour to 5 hours after administration, at least six components were identified conclusively:

1)the ibuprofen parent form
2)the w-oxidative form
3)the w-1 oxidative form
4)the parent-glucuronate
5)the w-oxidative form-glucuronate
6)the w-1 oxidative form-glucuronate.

A compound structure for each metabolite was confirmed by comparison of the experimentally determined accurate mass and isotopic ratios with theoretical values .

Furthermore, LC/MS data of urine samples from five people were analyzed using multivariate analysis by GeneSpringMS software. For both exogenous and also endogenous compounds, these increase and decrease profiles were analyzed.

(Detailed in ASMS 2009
Poster #584 ,Maeda, Kogure, Ishii)

Experimental

Administration
Tablets of a cold medicine containing ibuprofen- "Corgen Kowa IB Capsule"(two capsules, total 90 mg ibuprofen) were administrated to five male adults between the ages of 30 to 50.

Sampling
Urine samples were taken on a time basis, first prior to administration (0 hr) and then at 0.5 ,1, 2, 3, 4 and 5 hour intervals post-administration.

Preparation
Each urine sample was prepared by diluting 1/5 using distilled water before Q-TOF and QQQ LC/MS analysis.
Urine samples which appeared thick or contained precipitates were filtered using Ultrafree-MC 2.5 µm filters (Millipore) by centrifuging for 10 minutes at 10,000 rpm.

For the semi-quantitative analysis using a QQQ LC/MS system, 200 µL of 100 ng/mL ketoprofen (internal standard) aqueous solution was mixed with 100 µL of urine sample, and ultra pure water was added to take the sample volume to 500 µL.

QQQ and Q-TOF LC/MS Analysis
Mass spectrometer
Agilent 6460 QQQ LC/MS System
Agilent 6530 Q-TOF LC/MS System

Mass spectrometer ionization
ESI equipped with Agilent Jet Stream technology
Gas Temp 350 °C
Gas Flow 12 L/min
Nebulizer 60 PSI
Sheath Gas Temp 400 °C
Sheath Gas Flow 12 L/min

HPLC System
Agilent 1200 SL Binary Pump,
Agilent 1200 Column Oven,
Agilent 1200 Well Plate Autosampler SL Plus

Column
ZORBAX Eclipse Rapid Resolution HT 3.0 x 100 mm, 1.8µm

A soln 10mM ammonium acetate/Distilled water
B soln Acetonitrile

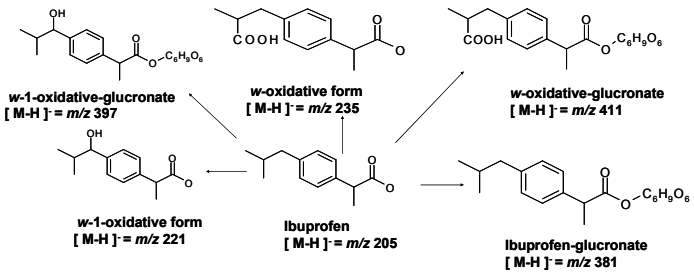
Gradient

Time(min.)	B conc. (%)
0	5
1.0	5
7.0	90
8.0	90

Results and Discussion

MRM analysis design

Target analysis for ibuprofen metabolites was designed on the basis of well-known metabolite forms and the result of accurate mass data analysis by the Q-TOF mass spectrometer and Metabolite ID software.



Structures of ibuprofen and its major metabolites

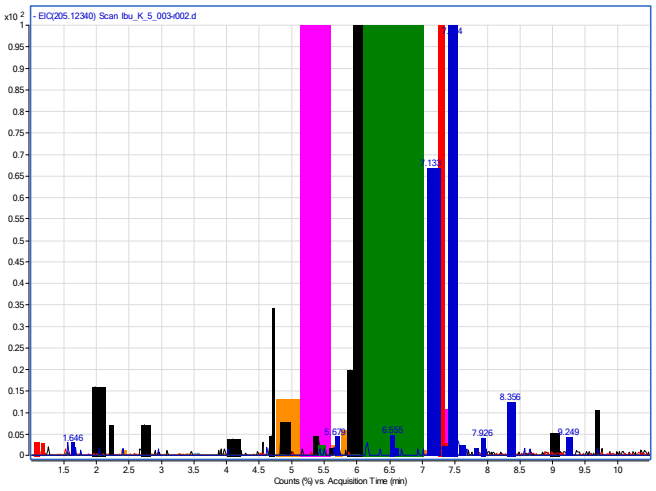
Yamamoto et al. "Dictionary of Drug Metabolism" (Hirokawa Publishing Co.)

Q-TOF analysis and Metabolite ID

No obvious differences or features were found between each Q-TOF LCMS total ion chromatogram of samples collected at 0, 0.5, 1, 2, 3 4, 5 hours after administration.

Predicting and exploring for possible metabolite molecules were done using MetaboliteID Software (Agilent Technologies). MetaboliteID software calculated the molecular weight of the possible metabolites based on the formula of ibuprofen. Confirmation of the metabolite structures was done using a combination of multiple algorithms such as 1) Isotopic pattern matching based on the *m/z* value acquired by LC/TOF analysis 2) , mass defect analysis which detects the accuracy of the fractional portion of the *m/z* value.

The results of the survey by MetaboliteID software suggested the presence of metabolites produced by biotransformations such as hydroxylation, glucuronidation + hydroxylation, demethylation, taurine conjugation, etc.



Extract ion chromatograms of the predicted metabolite molecules

QQQ MRM optimization

Determination of appropriate MRM transitions and the optimization of parameters such as fragmentor voltage (V) or collision energy for the predicted metabolites was conducted by Optimizer software (included with the system software).

For ibuprofen and ketoprofen (IS), a standard solution was used to optimize the parameters. However, as there are no commercially available standards for the predicted metabolite molecules, a urine sample was repeatedly analyzed using the Optimizer software to set the parameters for those metabolites.

Metabolites Browser

Short Summary | <Filtered>

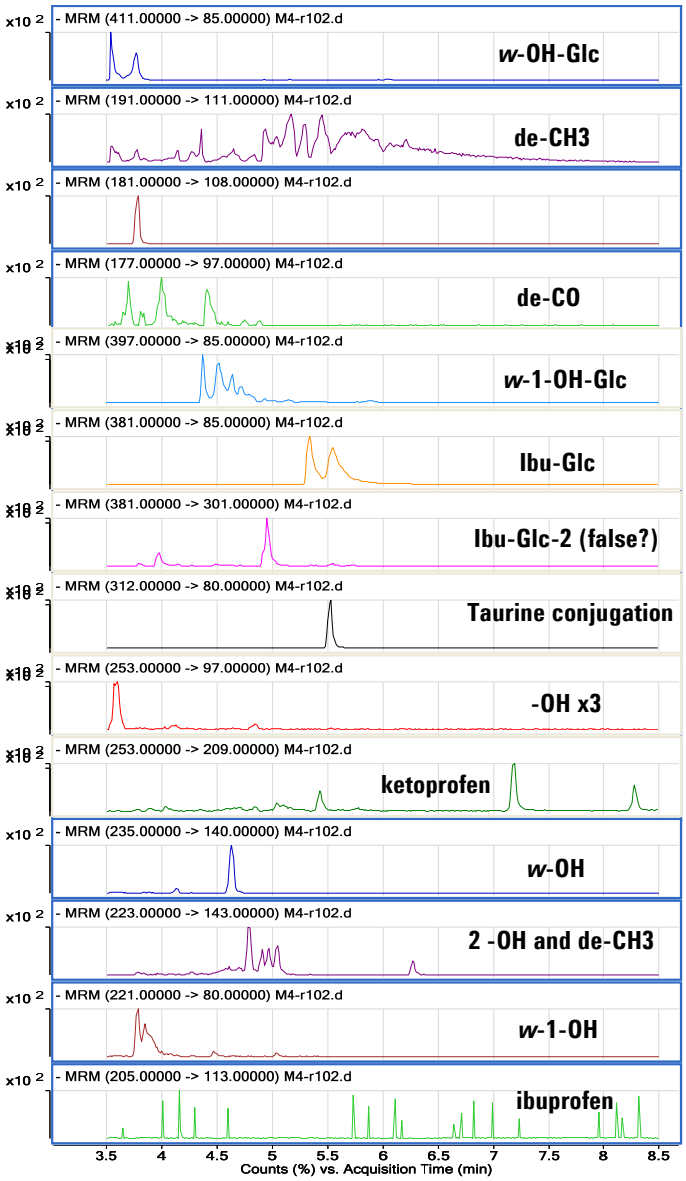
Metabolites						Sample Co...	Mass Def...	Isotopic Pa...	Biotransformations
Warnings	Name	RT	Mass	m/z	Rele...	Qualified	Qualified	Qualified	Assigned
1	Hydroxylation + Glucuronide	6.459	398.1598	397.1525	75.0	☑	☑	☑	☑
2	Hydroxylation + Glucuronide	6.545	398.1591	397.1518	75.0	☑	☑	☑	☑
3	Hydroxylation + Glucuronide	5.398	398.1561	397.1489	75.0	☑	☑	☑	☑
4	Taurine Conjugation	7.310	313.1344	312.1271	75.0	☑	☑	☑	☑
5	3x Hydroxylation	5.988	254.1158	253.1085	50.0	☐	☑	☑	☑
6	Hydroxylation and Ketone Formation	5.196	236.1062	235.0989	75.0	☑	☑	☑	☑
7	Demethylation and two Hydroxylations	5.208	224.1057	223.0984	75.0	☑	☑	☑	☑
8	Hydroxylation	6.242	222.1269	221.1196	75.0	☑	☑	☑	☑
9	Demethylation	5.166	192.1163	191.1090	75.0	☑	☑	☑	☑
10	Demethylation	4.996	192.1158	191.1085	75.0	☑	☑	☑	☑
11	Decarbonylation	6.242	178.1365	177.1292	75.0	☑	☑	☑	☑

Predicted ibuprofen metabolites in the urine sample collected at 5 hours after administration .

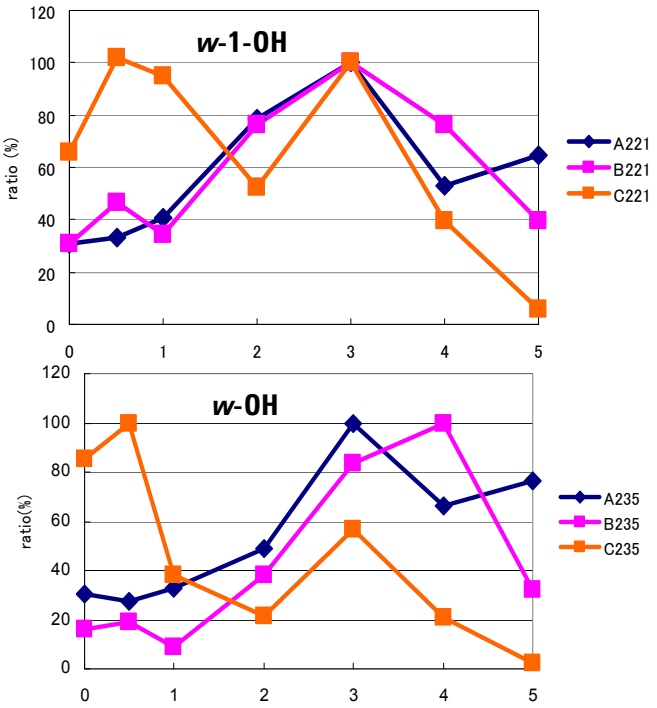
Results and Discussion

Compound Name	Precursor Ion	Product Ion	Dwell (msec)	Fragmentor (eV)	Collision Energy (eV)	Polarity
w-OH-Glc	411	85	50	80	25	Negative
w-1-OH-Glc	397	85	50	80	30	Negative
Ibu-Glc	381	301	50	80	25	Negative
Ibu-Glc 2	381	85	50	80	25	Negative
Taurine conj.	312	80	50	80	25	Negative
Ketoprofen(IS)	253	209	50	80	10	Negative
3x -OH	253	97	50	80	25	Negative
w-OH	235	140	50	80	25	Negative
2 -OH and de-CH3	223	143	50	80	25	Negative
w-1-OH	221	80	50	80	30	Negative
Ibuprofen	205	113	50	80	25	Negative
de -CH3	191	111	50	80	25	Negative
de- CO	177	97	50	80	25	Negative

MRM transitions and parameters for detection of ibuprofen metabolites



The extracted ion MRM chromatograms of the ibuprofen metabolites



Time course of metabolite amount in urine samples of three individuals: A (blue) , B (pink) , C (orange). *w*-1-OH (top) / *w*-OH (bottom). Vertical axis is the ratio (%) to the most abundant sample (100%) in the series.

QQQ MRM analysis

Using ketoprofen as an internal standard, the ibuprofen parent form and 11 metabolites were monitored to determine the relative change with time course. With this method, *w*-1-OH and *w*-OH (shown in figure above), and *w*-OH-Glc, *w*-1-OH-Glc, Ibu-Glc increased in relative amount, with the peak occurring around 3 hrs after the administration. Only a slight change of ibuprofen parent concentration was detected in these urine samples, which agrees with the well-known behavior of ibuprofen in the human body.

Conclusions

- Semi-quantitative analysis of the ibuprofen metabolites by Agilent 6460 QQQ LC/MS was possible using ketoprofen as an internal standard.
- The ibuprofen metabolites in human urine were readily identified using a combination of accurate mass analysis and specialized metaboliteID software.