Identification of the Microbial Fermentation Products for Curcumin using Metabolite ID Workflow on High Resolution Mass Spectrometry

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INTRODUCTION

Microbial fermentation of traditional Chinese medicine (TCM) has been a new focus in TCM research. Metabolic pathway profiling of active ingredients in TCM during microbial fermentation provides a new way for potential active compound screening. High Resolution mass spectrometry is a superior analytical technique for metabolite profiling and unknown identification.

In this report, we used curcumin as a model compound and described a rapid analytical strategy to perform metabolic pathway profiling of active TCM component using high resolution mass spectrometry system.

By using real time mass defect filter (MDF)/dynamic background subtraction (DBS) -Information dependent MS/MS acquisition, a total of 22 metabolites for curcumin were identified based on acquired accurate MS and MS/MS data in a single injection.

MATERIALS AND METHODS

HPLC Conditions:
A Shimadzu Prominence LC system with a Waters Symmetry C18, 150x2.1mm, 5μm at 40°C with a gradient of eluent A acetonitrile and eluent B water + 2mM ammonium acetate + 0.05% formic acid was used at a flow rate of 400μL/min. The injection volume was set to 3μL.

MS/MS Conditions:
An AB SCIEX TripleTOF® 4600 system with DuoSpray™ ion source and Electrospray Ionization (ESI) probe was used.
TOFMS
–ESI+/- ionization with DuoSpray ion source
–TOF mass/charge range: m/z = 100 -1200
–200 ms accumulation time
IDA Selection Criteria
–Dynamic Background Subtraction (DBS) & Multiple Mass Defect filters (MDF) were set as preferred IDA criteria
IDA MS/MS
– 8 Dependent scans, 100 ms each
– TOF mass/charge range: m/z = 50 to 1200

Workflow for metabolite identification

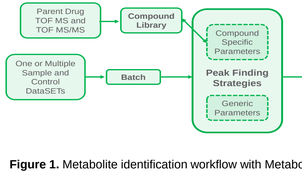
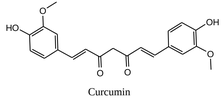


Figure 1. Metabolite identification workflow with MetabolitePilot™



Curcumin

Figure 2. Structures of Curcumin

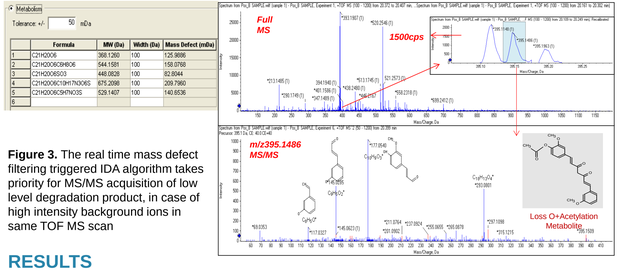


Figure 3. The real time mass defect filtering triggered IDA algorithm takes priority for MS/MS acquisition of low level degradation product, in case of high intensity background ions in same TOF MS scan

RESULTS

MetabolitePilot™ software for data processing, metabolites for curcumin were identified and tentatively characterized based on their retention times, accurate mass measurement of molecular and fragment spectrum (Fig. 4~5).

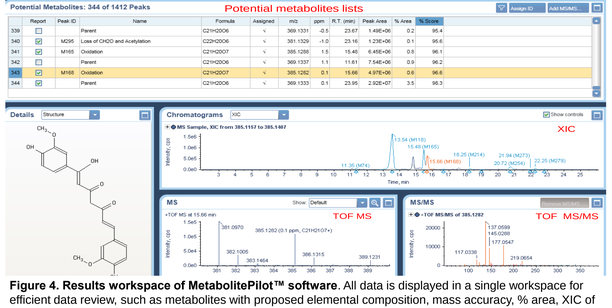


Figure 4. Results workspace of MetabolitePilot™ software. All data is displayed in a single workspace for efficient data review, such as metabolites with proposed elemental composition, mass accuracy, % area, XIC of metabolites, TOF MS and MS/MS, with scoring information

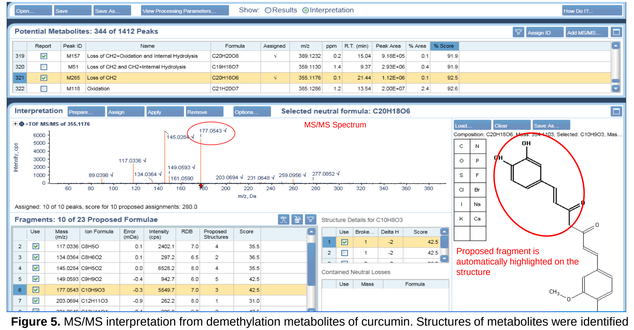


Figure 5. MS/MS interpretation from demethylation metabolites of curcumin. Structures of metabolites were identified on the basis of the high resolution MS/MS fragmentation using MetabolitePilot™ software. Structure editing and automatic fragment assignment can be performed for a proposed metabolite. The selected fragments are assigned and highlighted on the chemical structure.

Table 1. Curcumin metabolites detected in microbial fermentation.

Peak ID	Biotransformation	Formula	m/z [M+H]+	ppm	R.T. (min)	Peak Area	% Area
M1	Oxidation and Methylation	C22H22O7	399.1439	0.1	12.9	549000	1.9
M2	Loss of CH2+Internal Hydrolysis	C20H20O7	373.1288	1	13.7	170000	0.6
M3	Loss of CH2+Oxidation and Internal Hydrolysis	C20H20O8	389.1232	0.2	15	918000	3.1
M4	Oxidation	C21H20O7	385.1288	0.6	15.5	6450000	22.1
M5	Oxidation	C21H20O7	385.1282	0.6	15.7	4970000	17
M6	Demethylation to Carboxylic Acid	C21H18O8	399.1074	-0.5	16.1	415000	1.4
M7	Loss of CH2O+Internal Hydrolysis	C20H20O7	357.1331	-0.7	18.5	148000	0.5
M8	Loss of CH2+Internal Hydrolysis	C20H20O7	373.1282	0	18.6	200000	0.7
M9	Loss of CH2+Acetylation	C22H20O7	397.1275	-0.8	18.9	662000	2.3
M10	Internal Hydrolysis	C21H22O7	387.1435	-0.7	19.8	737000	2.5
M11	Loss of O and Acetylation	C23H22O6	395.1476	-0.6	20.4	86500	0.3
M12	Internal Hydrolysis	C21H22O7	387.1436	-0.2	20.5	268000	0.9
M13	Loss of CH2	C20H18O6	355.1176	0.1	21.4	1120000	3.8
M14	Oxidation	C21H20O7	385.1276	-1	22.3	412000	1.4
M15	Loss of CH2O and Acetylation	C22H20O6	381.1328	-0.9	22.8	1050000	3.6
M16	Loss of CH2O and Acetylation	C22H20O6	381.1329	-0.6	23.2	1230000	4.2
M17	Methylation	C22H22O6	383.1476	-0.3	23.2	149000	0.5
M	Parent	C21H20O6	369.1333	0.1	24	29200000	100
M18	Loss of CH2 and Acetylation	C22H20O6	381.133	-0.4	24	640000	2.2
M19	Loss of CH2+Acetylation	C22H20O7	397.1279	-0.3	24.2	633000	2.2
M20	Oxidation and Methylation	C22H22O7	399.1439	0.2	24.4	238000	0.8
M21	Loss of CH2O and Acetylation	C22H20O6	381.1331	-0.6	24.4	1370000	4.7
M22	Loss of CH2O and Acetylation	C22H20O6	381.1328	-1	24.7	1110000	3.8

The major metabolic pathway was found to be oxidation and a number of demethylation or hydroxymethylene loss combined acetylation metabolites.

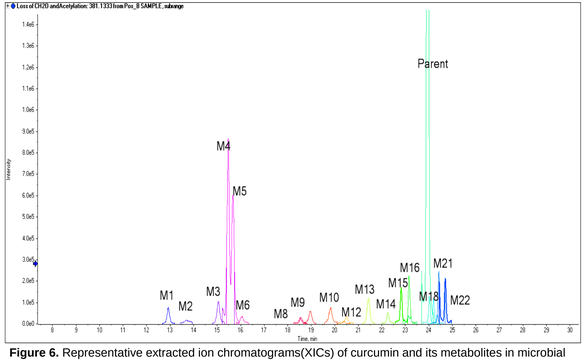


Figure 6. Representative extracted ion chromatograms(XICs) of curcumin and its metabolites in microbial fermentation. Major metabolites are labeled.

CONCLUSIONS

With only one injection, real time MDF/ DBS-IDA method was able to effectively trigger MS/MS acquisition for all major curcumin metabolites, even some of these are low levels with very weak spectrum in full-scan. Superior mass accuracy for both TOF MS and MS/MS spectra effectively eliminated many false-positives and improved the confidence for identification. For data processing, chromatographic peaks of all major metabolites can be quickly identified and the corresponding structure for parent and metabolites was easy elucidated by MetabolitePilot™ software. Finally, the major metabolic pathway during microbial fermentation process was found to be oxidation, demethylation and acetylation etc. A new workflow is established for TCM microbial fermentation products was developed.

TRADEMARKS/LICENSING