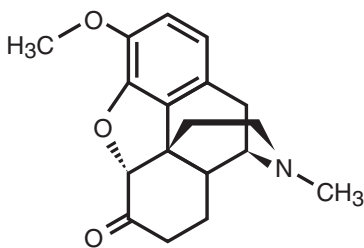


# **LC/MS/MS Bioanalysis of Acidic and Basic Compounds with Double Liquid-Liquid Extraction by Changing pH in Between**

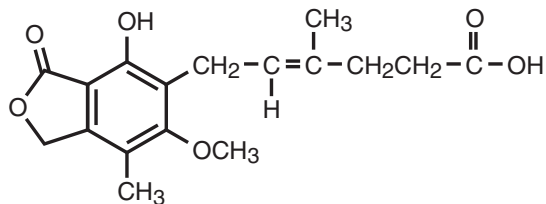
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Hydrocodone (HCD)



Mycophenolic acid (MPA)

## Introduction

- Sample clean-up is very important for bioanalytical analysis
- Liquid-liquid extraction is widely used: clean, fast, low cost
- Different chemicals are extracted at different conditions
- Liquid-Liquid extraction at basic condition for HCD
- Liquid-Liquid extraction at acidic condition for MPA
- What is the extraction method to quantitate both compounds from one sample?
- **Double liquid-liquid extraction by changing pH in between**
- Tomtec automation
- Positive-negative ion switch during detection

## Method

### Extraction procedure (Double liquid-liquid extraction)

- Add 100  $\mu$ L sample to sample plate and 50  $\mu$ L IS (HCD-d6 and MPA-d3)
- Add 50  $\mu$ L saturated borate
- Add 650  $\mu$ L MTBE
- Vortex, centrifuge, and transfer organic to a clean plate then blow down to dryness
- Add 50  $\mu$ L 10% formic acid to the same sample plate
- Add 650  $\mu$ L MTBE
- Vortex, centrifuge, and transfer organic to the same extract plate then blow down to dryness
- Reconstitute the extract plate and inject on LC/MS/MS

### HPLC conditions

Column:	Gemini C18, 50 x 2.0, 3 $\mu$ , #408886-5	
Mobile Phase A:	0.1% formic acid in water	
Mobile Phase B:	100% Acetonitrile	
Run time:	7.5 minutes	
Retention time:	2.5 minutes for HCD; 4.2 minutes for MPA	
Gradient:	<u>Time (min)</u>	<u>%B</u>
	0	5
	1	5
	3	50
	5	82
	5.05	90
	6.5	90
	6.55	5

### Tandem mass spectrometry

Mass spectrometer:	API 4000
Source:	Turbo Ionspray
Resolution:	Unit/Unit
Ion Monitored:	0 to 3.2 minutes HCD and HCD-d6, +300.1/199, +306.2/202,
	3.2 to 6.0 minutes MPA and MPA-d3, -319/191, -322/191,

# Results

## Calibration standard statistics

### HCD

Assay Date	Analytical Run Number	STD 8 0.200 ng/mL	STD 7 0.400 ng/mL	STD 6 1.00 ng/mL	STD 5 5.00 ng/mL	STD 4 25.0 ng/mL	STD 3 50.0 ng/mL	STD 2 75.0 ng/mL	STD 1 100 ng/mL
28-Jan-2008	2	0.202	0.388	1.09	5.06	25	48.7	74.4	103
		0.202	0.38	0.985	5.01	24.7	49.1	74.3	101
Mean		0.202	0.384	1.04	5.04	24.9	48.9	74.4	102
%Bias		1	-4	4	0.8	-0.4	-2.2	-0.8	2
n		2	2	2	2	2	2	2	2

### MPA

Assay Date	Analytical Run Number	STD 8 50.0 ng/mL	STD 7 100 ng/mL	STD 6 250 ng/mL	STD 5 1250 ng/mL	STD 4 6250 ng/mL	STD 3 12500 ng/mL	STD 2 18800 ng/mL	STD 1 25000 ng/mL
28-Jan-2008	2	49.7	98.9	248	1260	6070	12200	18800	25300
		50.1	102	249	1290	6230	12300	18800	25200
Mean		49.9	100	249	1280	6150	12300	18800	25300
%Bias		-0.2	0	-0.4	2.4	-1.6	-1.6	0.3	1.2
n		2	2	2	2	2	2	2	2

## QC statistics

### HCD

Run Date	Curve Number	LLOQ 0.200 ng/mL	QC L 0.600 ng/mL	QC M 35.0 ng/mL	QC H 75.0 ng/mL
28-Jan-2008	2	0.21	0.576	35.1	76.4
		0.215	0.59	35.4	77.1
		0.196	0.586	35.1	76.2
		0.194	0.619	34.9	76.2
		*0.737	0.607	34.9	76.2
		0.205	0.57	35.6	77.8
Mean		0.204	0.591	35.2	76.7
S.D.		0.00897	0.0186	0.28	0.663
%CV		4.4	3.1	0.8	0.9
%Theoretical		102	98.5	100.6	102.3
%Bias		2	-1.5	0.6	2.3
n		5	6	6	6

### MPA

Run Date	Curve Number	LLOQ 50.0 ng/mL	QC L 150 ng/mL	QC M 8750 ng/mL	QC H 18800 ng/mL
28-Jan-2008	2	48.9	153	8810	19600
		47.2	153	8740	19500
		49.2	150	8760	19100
		48.9	154	8630	19600
		48.6	155	8850	19400
		45.8	152	8740	19300
Mean		48.1	153	8760	19400
S.D.		1.33	1.72	75	194
%CV		2.8	1.1	0.9	1
%Theoretical		96.2	102	100.1	103.5
%Bias		-3.8	2	0.1	3.5
n		6	6	6	6

Reason Deactivated

\* M - QC is a statistical outlier and can be dropped per BASi SOPs

## Selectivity

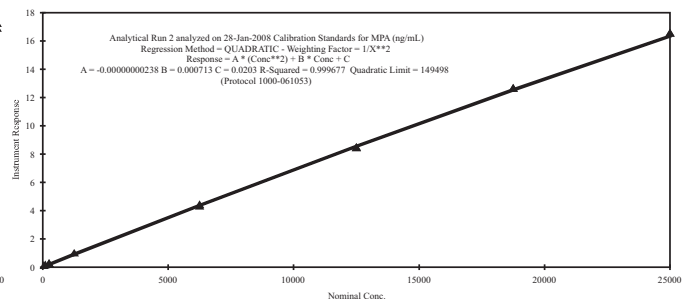
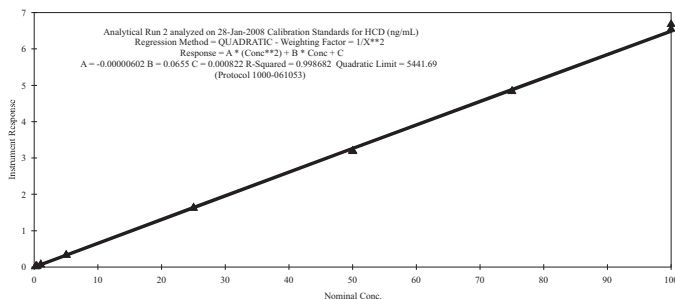
### HCD

Vendor	Lot #/ID	Analyte Peak Area	Analyte Response Relative to LLOQ	ISTD Peak Area	ISTD Response Relative to LLOQ
Biochemed	R22187	0	0.0%	0	0.0%
Biochemed	R22181	106.896119	2.5%	0	0.0%
Biochemed	R22186	122.934678	2.8%	0	0.0%
Biochemed	R22185	82.3330364	1.9%	0	0.0%
Biochemed	R22167	71.7682354	1.7%	0	0.0%
Biochemed	R22182	84.4247512	1.9%	0	0.0%
	Low Calibrator	4742.12645		336960.64	
	Low Calibrator	3927.4293		278923.496	

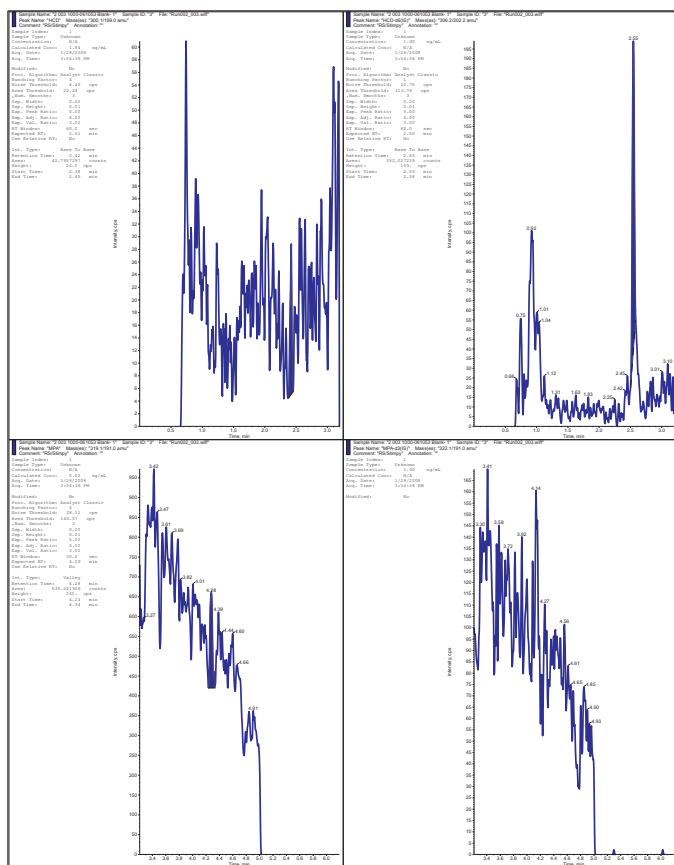
### MPA

Vendor	Lot #/ID	Analyte Peak Area	Analyte Response Relative to LLOQ	ISTD Peak Area	ISTD Response Relative to LLOQ
Biochemed	R22187	0	0.0%	0	0.0%
Biochemed	R22181	0	0.0%	0	0.0%
Biochemed	R22186	0	0.0%	0	0.0%
Biochemed	R22185	0	0.0%	0	0.0%
Biochemed	R22167	0	0.0%	0	0.0%
Biochemed	R22182	0	0.0%	0	0.0%
	Low Calibrator	31110.2708		522076.255	
	Low Calibrator	30576.2754		545669.137	

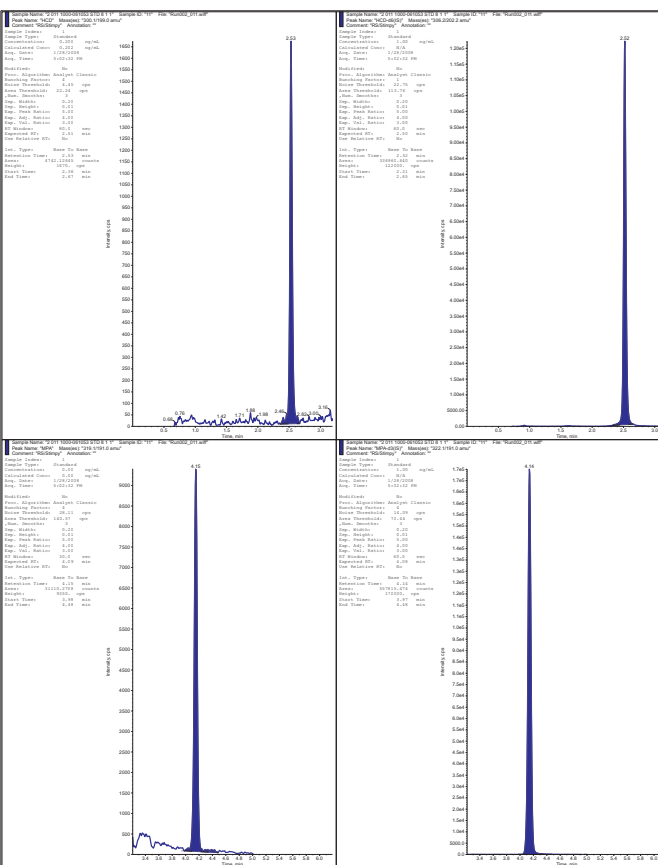
## Representative calibration curve



## Typical chromatogram Blank matrix



## Low calibration standard 0.200 ng/mL for HCD and 50.0 ng/mL for MPA



## Discussion

The recovery for liquid-liquid extraction is variable with different extraction conditions.

	Liquid-Liquid extraction recovery at different pH conditions:	
	Recovery (%)	
	HCD	MPA
Double extraction (basic, then acidic condition)	75.0	69.5
	71.8	69.4
L-L extraction at basic condition only	52.2	0.2
	49.9	0.2
L-L extraction at acidic condition only	0.4	49.0
	0.4	48.6

The presence of drugs, their metabolites and co-administered drugs often require quantitation of a set of chemicals with different chemical properties, such as pKa. One solvent or pH extraction condition could prove difficult in achieving adequate recovery for all the interested compounds in a single extraction. Double liquid-liquid extraction gives the flexibility to change extraction pH, as demonstrated above, or extraction solvents to greatly enhance analyte recovery.

## Conclusion

This novel methodology enlarges the scope of a liquid-liquid extraction sample preparation method. The method could be adjusted according to analytes' pKa. Acidic, basic or neutral conditions could be combined to extract compounds with different chemical identities, such drug and metabolites.