SUPPLEMENTARY INFO

Table 2
Exogenous interferences that were investigated by fortification of 1000 ng/mL of each compound into blank urine and LOD samples, respectively

11-hydroxy-tetrahydrocannabinol	imipramine
2C-B	ketamine
6-acetylcodeine	lorazepam
6-acetylmorphine	methadone
7-aminoclonazepame	methamphetamine
7-aminoflunitrazepam	methylbenzodioxolylbutanamine (MBDB)
7-aminonitrazepam	methylendioxyamphetamine (MDA)
alprazolam	methylendioxyethylamphetamine (MDEA)
amphetamine	methylendioxymethamphetamine (MDMA)
anhydroecgonine methyl ester	m-hydroxybenzoylecgonine
acetylsalicylic acid	m-hydroxycocaine
benzodioxolylbutanamine (BDB)	morphine
benzoylecgonine	morphine-3-glucuronide
bromazepam	morphine-6-glucuronide
brompheniramine	nicotine
buprenorphine	nitrazepam
caffeine	norbenzoylecgonine
cannabidiol	norbuprenorphine
cannabigerol	norcocaethylene
cannabinol	norcocaine
chlorpheniramine	norcodeine
clomipramine	norcotinine
clonazepam	nordiazepam
clonidine	norfluoxetine
cocaethylene	normorphine
cocaine	noroxycodone
codeine	noroxymorphone
cotinine	oxazepam
dextromethorphan	oxycodone
diazepam	oxymorphone
dihydroxymethamphetamine (HHMA)	parexetine

diphenhydramine	pentazocine
ecgonine ethyl ester	phencyclidine
ecgonine methyl ester	phentermine
EDDP	phenylpropanolamine
EMDP	p-hydroxybenzoylecgonine
ephedrine	p-hydroxycocaine
ethylamphetamine	p-methoxyamphetamine (PMA)
flunitrazepam	p-methoxymethamphetamine (PMMA)
fluoxetine	propoxyphene
flurazepam	pseudoephedrine
hydrocodone	cathinone
hydromorphone	temazepam
hydroxyamphetamine	tetrahydrocannabinol (THC)
hydroxycotinine	tetrahydrocannabinol carboxylic acid
hydroxymethamphetamine	tetrahydrocannabinol carboxylic acid glucuronide
hydroxymethoxyamphetamine (HMA)	tetrahydrocannabinol glucuronide
hydroxymethoxymethamphetamine (HMMA)	tetrahydrocannabinolic acid A (THCA-A)
ibuprofen	tylenol

Table 3

Results of the hydrolysis optimization study with four replicates showing the peak areas in the non-hydrolyzed samples and the mean percentage of the peak area ratio of hydrolyzed to non-hydrolyzed samples. The hydrolysis procedure was varied starting from the initial conditions (#1). Optimal conditions, which were used for all later analysis, were: incubation for 2 h at 55 °C with 2000 units enzyme at pH 4.0 (#2).

* irregular peak shape, precise integration not possible

[1] significantly superior compared to the initial conditions

[2] not significantly different compared to the approach with 4000 units enzyme

	non-hydrolyzed samples	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**
Duration (hours)	-	1	2	0.5	1	1	1	1	1	1
Enzyme (units)	-	2000	2000	2000	1000	4000	2000	2000	2000	2000
Temperature (°C)	-	55	55	55	55	55	37	70	55	55
pH of buffer	-	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0	6.0
	Peak area	**Percentage of peak area ratios hydrolyzed/non-hydrolyzed set**								
JWH-018 5-OH-pentyl glucuronide	9.13E+06	1.1%	0.8%	3.2%	1.2%	0.8%	4.1%	2.1%	1.5%	33.9%
AM2201 4-OH-pentyl	4.17E+06	222%	225%	190%	211%	228%	170%	214%	223%	117%
AM2201 6-OH-indole	2.66E+06	269%	271%	249%	274%	256%	231%	264%	270%	140%
JWH-018 pentanoic acid	1.91E+07	99%	101%	101%	104%	98%	101%	96%	97%	*
JWH-018 OH-indole	1.49E+04	140%	131%	130%	135%	130%	135%	136%	137%	115%
JWH-018 5-OH-pentyl	2.43E+07	145%	148%	144%	149%	144%	143%	145%	147%	128%
JWH-073 butanoic acid	4.22E+06	104%	100%	104%	105%	103%	102%	103%	103%	96%
JWH-122 5-OH-pentyl	5.88E+05	205%	208%	204%	208%	206%	190%	189%	191%	134%
JWH-210 OH-pentyl	1.35E+03	908%	954%	677%	807%	924%	520%	909%	911%	309%
JWH-210 5-OH-indole	1.54E+03	480%	459%	463%	481%	477%	453%	475%	522%	350%
JWH-250 OH-pentyl	9.11E+04	2193%	2322% [2]	1496%	1803%	2484% [1]	1135%	2322%	2234%	354%

JWH-250 pentanoic acid	1.67E+05	96%	93%	95%	95%	90%	100%	89%	100%	58%
JWH-250 5-OH-indole	1.34E+05	201%	196%	198%	199%	200%	201%	192%	203%	174%
RCS-4 pentanoic acid	4.08E+03	442%	422%	416%	411%	371%	302%	426%	423%	133%