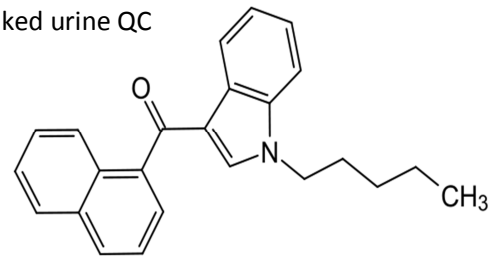
Determination of Spice in Urine by LC-on-line- SPE-MS/MS by using Spark Holland Symbiosis™ Pico system and LGC LoGiCal® standards.

Introduction:

Synthetic cannabis is a psychoactive designer drug derived of natural herbs sprayed with synthetic chemicals that, when consumed, allegedly mimic the pleasurable effects of cannabis. It is best known by the brand names K2 and Spice, both of which have largely become generalized trademarks used to refer to any synthetic cannabis product. (It is also for this reason that synthetic cannabis is often referred to as spice product, due to the latter.) .
There is controversy among calling Spice and K2 synthetic cannabis, since none of the chemicals contained in the products are copies of anything found in cannabis. Text from Wikipedia

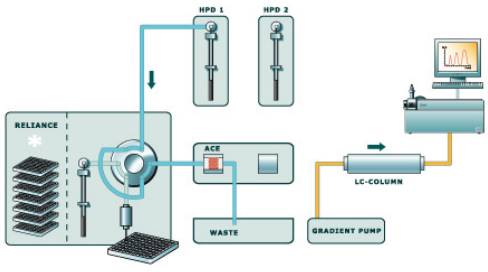
This application note describes the method development for the determination of 5 Spice compounds and the creation of calibration curves and measurement of spiked urine QC samples.
Note that no internal standard is used for this application.
The measured Spice compounds are:



- JWH-015
- JWH-081
- JWH-098
- JWH-122
- JWH-203

Symbiosis Pico.

Symbiosis Pico is an integrated (U)HPLC and Online SPE extraction system. It can clean-up, pre-concentrate, and process untreated samples fully automated. Online SPE and LC elution take place simultaneously, resulting in zero Sample Prep time. Also, with online SPE, 100% of the extracted sample is injected on to the HPLC column, resulting in highest possible sensitivity. The system is standard delivered with a fixed tubing layout which makes it possible to switch between multiple operating modes. The system can operate in different analytical modes: LC mode (system works as a standard (U)HPLC system), XLC mode (online SPE mode) and mXLC (special multi-dimensional online SPE mode).



Method.

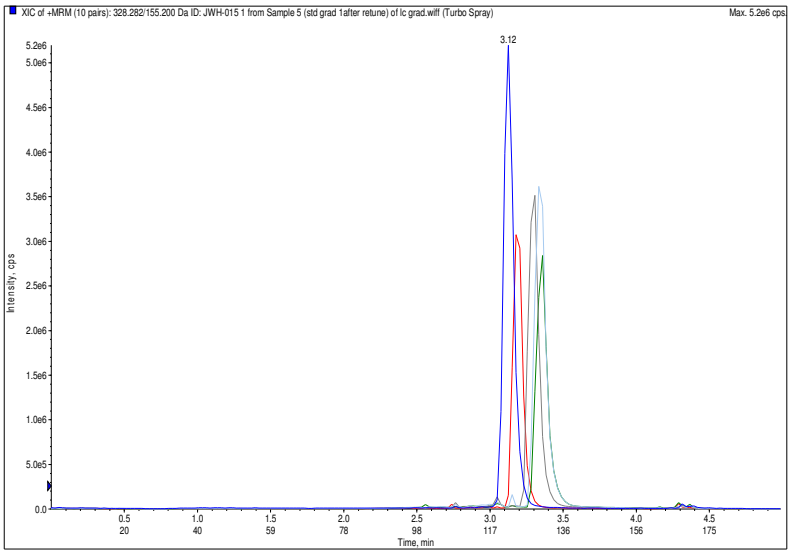
The Synthetic cannabis have a basic behavior in solution. Therefore the SPE method is developed under acidic conditions; all used SPE solvents contain 0.2% Formic acid (FA).
The method development started with the screening of the 6 reversed phase sorbents in the Method Development cartridge tray (Spark p/n:0822.660). After selecting the best cartridge sorbent the method was optimized by increasing the organic content in the wash solvent. After the method was developed urine samples are spiked and calibration curves and quality controls are measured.

Compounds.

The JWH-015, JWH-081, JWH-098, JWH-122 and JWH-203 are supplied as a gift from LGC Germany. (http://www.logical-standards.com/)

LoGiCal is a new range of reference materials from LGC, manufactured in house to the highest quality standards and certification levels at LGC's production facilities in Europe. The new range offers scientists a choice of solutions and powders prepared according to the principles of ISO Guide 34 – with the solutions being fully ISO Guide 34 certified – each supplied with its own detailed Certificate of Analysis.

The JWH-122, JWH-015 and JWH-203 are delivered in powder form (10 mg) the other two are dissolved in Acetonitrile (ACN) in a 1 mg/mL concentration. From dry compounds 1 mg is dissolved in 1 mL ACN to get a 1 mg/mL stock solution. 50µL of each compound in diluted with 5 mL 50% ACN in 0.2% FA to give an intermediate solution of 10µg/mL. From this intermediate solution all other dilutions are made by using 0.2% FA in water.

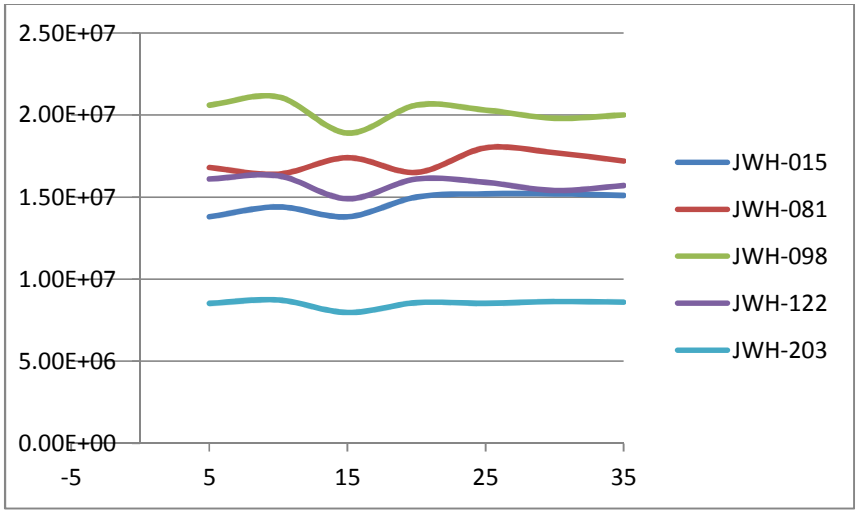


LC run: 5 µL 100 ng/mL mixture of 5 Spice compounds in 0.2% FA.

On-line SPE Method development.

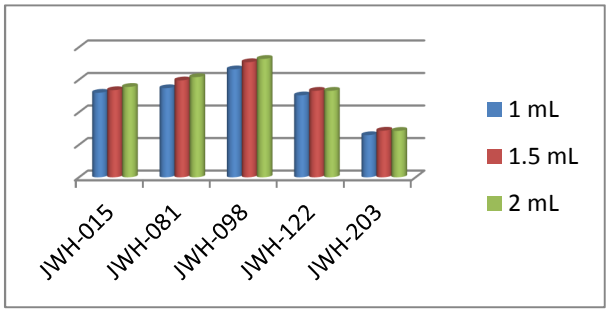
After the standard LC injection the 6 Spark HySphere reversed phase sorbents are tested using a neat solution, the sorbent screening procedure. The cartridge wash is performed with 0.2% FA in water without any organic. (data not shown)

The HySphere C8EC SE gives the best recovery (more than 95% for all compounds) and is selected to be used for this application. After the selection of C8EC SE as the sorbent of choice the application is further optimized.
First the highest percentage of organic in the wash determined. During the sorbent screening the cartridge was washed with water containing 0.2% Formic acid. The system is set up to automatically add 10%, 15%, 20%, 25%, 30% and 35% of ACN to the 0.2% FA to the wash solution.

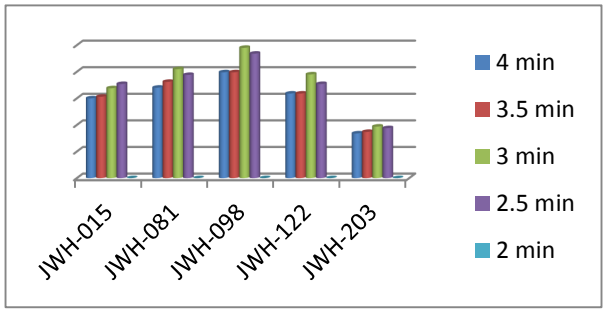


percentage ACN wash vs. the peak area.

After optimizing the wash percentage the next step is the cartridge wash volume optimization. The Symbiosis on-line SPE system can automatically modify the wash setting with different volumes; 1 mL, 1.5 mL and 2 mL. After the wash volume the shortest possible elution time is determined also in an automated routine.



wash volume



Elution time

The final on-line SPE (XLC) method is:

Cartridge:	data not shown HySphere C8EC SE (p/n:0822.611)
Solvation	1 mL 0.2% FA in ACN (5 mL/min)
Equilibration	1 mL 0.2% FA in 5% ACN (5 mL/min)
Sample application	1 mL 0.2% FA in 5% ACN (2 mL/min)
Wash 1	2 mL 0.2% FA in 30% ACN (5 mL/min)
Elution time	3 minutes LC gradiënt

Spark HOLLAND

Autosampler method

Injection Mode: Partial loop fill
Injection volume: 5 µL
Autosampler wash 1 mL 40% ACN in 0.2%FA

LC method

For the chromatography a Phenomenex Kinetex XB-C18 (2.6µ 50*2.1 mm) column is selected. **2.6 µm core-shell particles** operate at lower backpressures, giving chromatographers the ability to achieve sub-2 µm performance on ANY system, HPLC or UHPLC. Kinetex® core-shell particle technology takes an innovative approach to ultra-high performance. By growing a thin porous 'shell' over a solid silica core, chromatographers can achieve significant increases in efficiency, peak capacity, and resolution at pressures compatible with both HPLCs and UHPLCs alike.
(http://www.phenomenex.com/Kinetex/CoreShellTechnology)

Mobile phase A is 0.2% formic acid in water and mobile phase B is 0.2% formic acid in ACN.

Pump time	Pump flow (ml/min)	Pump Fraction A %	Pump Fraction B %
00:00:01	0.20	75	25
00:00:05	0.20	75	25
00:01:30	0.20	5	95
00:02:30	0.20	5	95
00:03:00	0.30	75	25
00:05:00	0.20	75	25

MS method

An AB Sciex API 4000 is used to detect the 5compounds
After tuning of the MS two most dominant fragments are selected.

	Q1	Q3	DP	CE	CXP
JWH-015 1	328	155	41	33	28
JWH-015 2	328	127	41	65	22
JWH-081 1	372	185	36	39	32
JWH-081 2	372	157	36	55	28
JWH-098 1	386	185	36	33	32
JWH-098 2	386	345	36	13	10
JWH-122 1	356	169	36	35	10
JWH-122 2	356	141	36	61	26
JWH-203 2	340	89.1	36	99	16
JWH-203 1	340	125	36	39	22

Compound depending settings

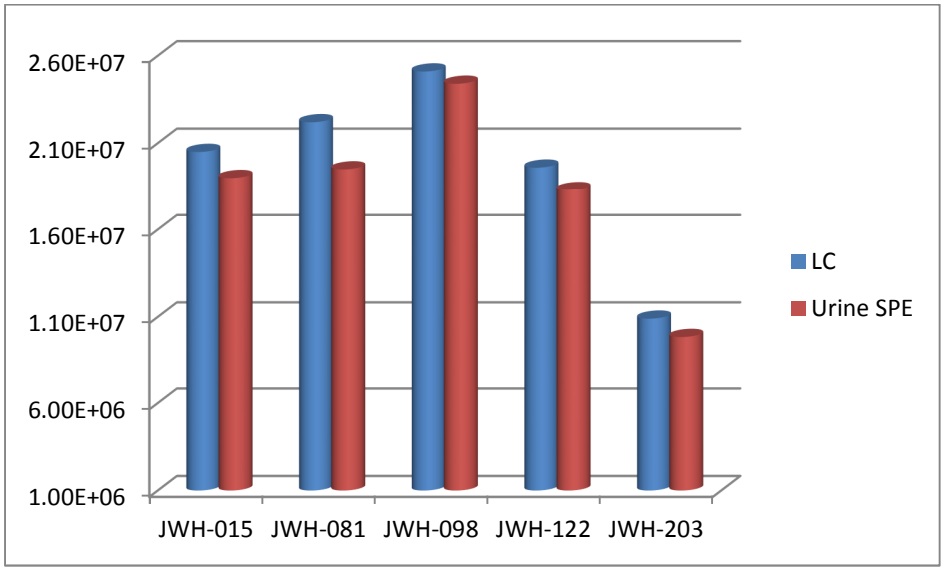
CAD	4
CUR	15
GS1	50
GS2	50
IS	5000
TEMP	450

Source and gas settings

Results.

A 100 ng/mL Spice standard is spiked in 0.2% FA in water and injected using the LC modes of the Symbiosis™ Pico system. This gives an indication of the expected signal height and area.

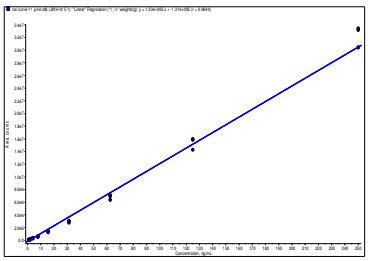
After the LC injection a spiked urine sample with the same concentration is loaded on the HySphere C8EC SE cartridge and processed using the optimized on-line SPE method. The recoveries are for all 5 compounds higher than 90%



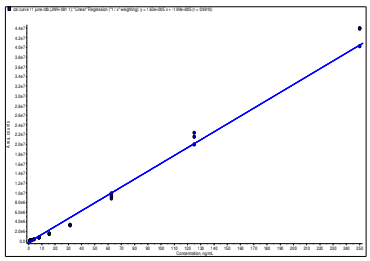
Recovery of the 5 spice compounds.

Calibration curve

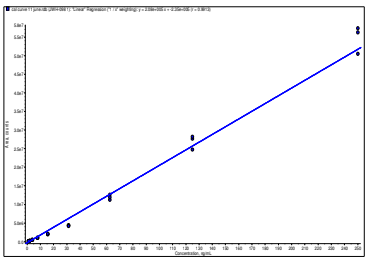
A calibration curves and QCs are prepared in urine. Starting with the intermediate solution the standards are prepared in urine. Every sample contained 50% urine and 50% 0.2% FA, this to resolve any pH differences between urine samples. The calibration curve ranges from 1 ng/ml to 250 ng/mL using 9 calibration points.



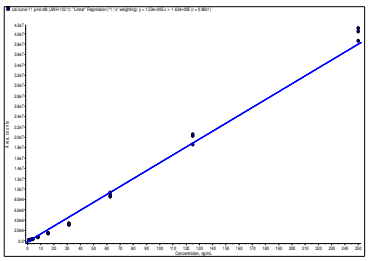
JWH-015



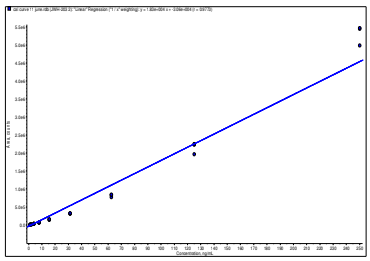
JWH-081



JWH-098



JWH-122



JWH-203

Remarks:

- All calibration curves have a R>0.99 with a 1/ X Weighting.
- No internal standard is used.
- The results are created by combining the data of 3 calibration curves.
- %CV values are less than 5% for all 5 compounds and all concentrations
- Accuracy is between 90 and 110% for all 5 compounds and all concentration.

QC

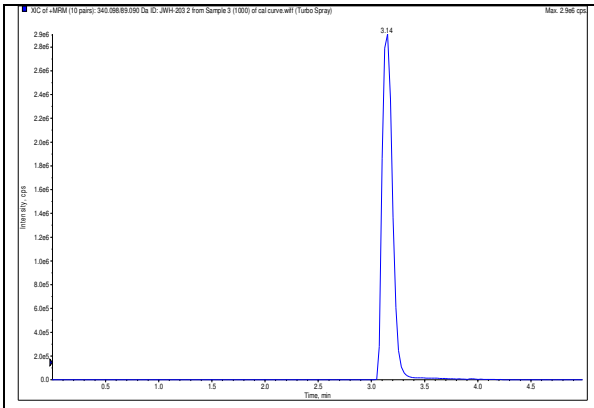
For the quality control standards new samples are prepared in urine with concentrations from 1 ng/ml, 100 ng/ml and 250 ng/mL. these are injected nine times, three samples after each of the three calibration curves.

	JWH-015	**JWH-015**	**JWH-081**	**JWH-081**	**JWH-098**	**JWH-098**	**JWH-122**	**JWH-122**	**JWH-203**	**JWH-203**
	%CV	*Accuracy*	*%CV*	*Accuracy*	*%CV*	*Accuracy*	*%CV*	*Accuracy*	*%CV*	*Accuracy*
1 ng/ml	0.95	115.2	1.53	110.6	1.32	107.2	1.70	113.0	0.91	108.0
100 ng/ml	6.61	105.2	7.10	105.2	6.97	102.5	6.84	105.0	6.13	103.2
250 ng/ml	7.51	95.3	10.54	91.8	11.35	100.0	11.41	92.9	10.26	101.5

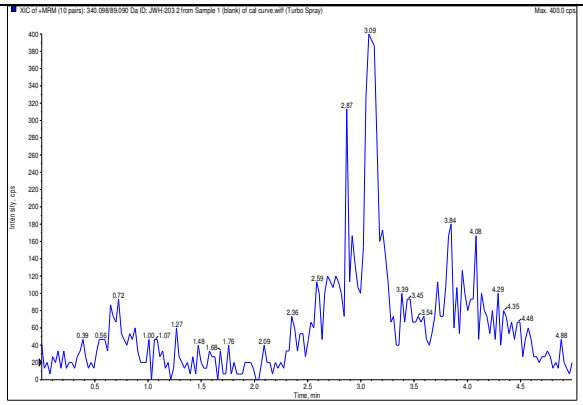
QC and accuracy values of the 5 spice compounds after nine injections

Carry-over.

After a 1000 ng/mL spiked urine sample a blank sample containing 50% urine and 50% 0.2% FA was injected. The system was washed with the standard 1 mL 40% ACN in 0,2% FA wash routine.



1000 ng/mL JWH-203 spiked urine sample.
Peak area = 2e7



blank after the 1000 ng/mL urine sample.
peak area = 2e3

The other 4 spice compounds have the same or less carry-over percentage.

Conclusion:

From this study it can be concluded that within a time frame of one day it is possible to develop a XLC-MS method for the determination of 5 spice compounds with a recovery of close to 100%.
No internal standard is used for this application.
For the 5 spice compounds in urine the calibration curve correlation coefficient is more than 0.99% with a 1/X weighting..
The % CV is less than 5% for all points of the calibration curve. The spiked urine samples show no matrix effect compared to the un-spiked LC injection.
The results acquired with on-line SPE are compatible with the currently used off-line SPE method.
Carry-over is less than 0.01% using the standard wash after a 1000 ng/mL spiked urine sample.

About on-line SPE from Spark

The Symbiosis technology for online SPE is based on pressure resistant high performance SPE cartridges that can be automatically exchanged. SPE solvent delivery is provided by propriatary High Pressure Syringe pumps for accurate delivery, independent of cartridge backpressure. The operating principle of the Symbiosis online SPE system is presented in a series of diagrams below. Symbiosis has two clamps for SPE cartridge enabling parallel operation of extraction and LC analysis. Valves taking care of switching solvent flow directions are not shown.

About LoGiCal from LGC

LoGiCal is a new range of reference materials from LGC, manufactured in house to the highest quality standards and certification levels at LGC's production facilities in Europe. The new range offers scientists a choice of solutions and powders prepared according to the principles of ISO Guide 34 – with the solutions being fully ISO Guide 34 certified – each supplied with its own detailed Certificate of Analysis. As well as native substances, there is a wide selection of stable isotope labeled materials for use with mass spectrometry and other analytical techniques. Initially focusing on the needs of forensic and clinical laboratories, the new product range provides access to a wide range of reference materials, including drugs of abuse, prescription drugs and drug metabolites.
The LoGiCal range is exclusively available from – and distributed globally by – LGC Standards, with stocks maintained locally at LGC Standards offices to eliminate delays due to the complex, lengthy import Procedures associated with the procurement of controlled substances.