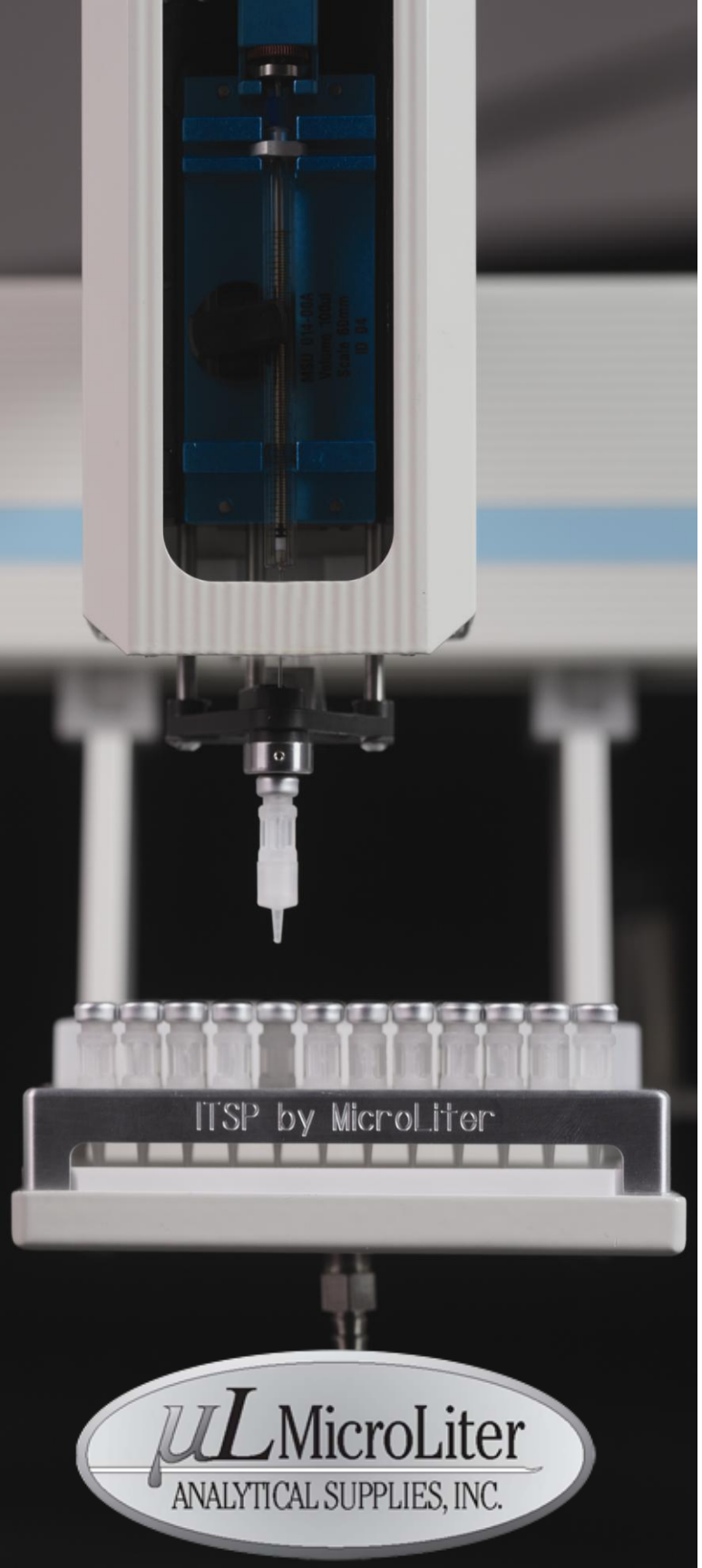

ITSP™

SAMHSA-5 Drugs of Abuse
Proof of Concept Application Notes

Automated Sample Prep

The SPE sample prep methods described in these application notes were developed using ITSP on CTC PAL autosamplers to take full advantage of sample prep automation. Benefits accrued include:

- Improved chain of custody
- Ease of inserting STAT samples in mid-run of assay
- Reduced risk of carryover
- Improved lab tech safety and productivity
- Increased quality of results
- Improved utility of capital equipment
- Reduced costs of consumables, solvent, disposal

LC/MS/MS

Many labs are moving to LC/MS/MS in order to perform more tests at lower cost in less time. LC/MS/MS offers a number of advantages versus the traditional analytical techniques of LC, GC, or GC/MS, including:

- Wider compound coverage
- Better sensitivity
- Better specificity
- Less sample prep

SAMHSA-5

The assays presented herein are to detect and quantify drugs of abuse in human urine.

- Cocaine (as BZE)
- Opiates
- Cannabinoids (as THCA)
- Amphetamines
- PCP

Coming soon!

New Application Notes with updated analytical results with HHS detection cutoff levels will be published soon.

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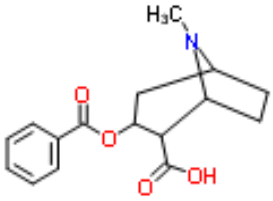
Determination of Benzoylecgonine in Human Urine Using Automated, In-Line, ITSP Solid Phase Extraction and Liquid Chromatography Mass Spectrometric Detection

Thurman L. Allsup[1], Kenneth C. Lewis[1] and Kim Gamble[2]
[1]OpAns, LLC, Durham NC, 27713, [2]Microliter Analytical Supplies, Inc., Suwannee GA, 30024

Abstract

A quantitative analytical method has been developed for the determination of benzoylecgonine (BZE) in human urine. BZE is the primary metabolite of cocaine found in urine and is the species used to confirm cocaine use (150 ng/mL confirmatory cutoff)[1]. Historical analytical procedures for the analysis of BZE in urine involve the extraction of BZE from the urine matrix followed by the evaporation of the eluant solvent and a multi-stepped derivatization procedure to prepare the sample for GC/MS. This is an extremely labor intensive procedure. The method that has been developed here is fully automated to quantitatively extract BZE from urine using C18 ITSP SPE devices and analyze the samples in-line by LC/MS/MS. The sample extraction and injection onto the LC/MS/MS were both performed in-line by a PAL (CTC) sample handling unit. Spiked urine samples with stable label internal standards were applied to C18 ITSP devices for extraction. The extracts were injected in-line onto an LC/MS/MS using a XDB-C18 (Agilent) column for separation. Selected reaction monitoring (SRM) was used for the analysis and a deuterated analog of the analyte was used for quantitation.



BZE
Exact Mass 289.1

Experimental

Sample Preparation

Benzoylecgonine standard and the deuterated analog were obtained from Cerilliant. A set of standards was prepared in human urine. Calibration standards were prepared at nominal concentrations of 25, 100, 250, 500, 1000, 2500 and 5000 ng/mL with a constant concentration of the deuterated analog at 500 ng/mL.

ITSP SPE Method

ITSP Cartridges: SPE µLplate w/C18 10mg (Product No.: 07-C1810-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

Step	Solvent	Volume (µL)	Flow Rate (µL/sec)
Condition	B	100	10
Condition	A	100	10
Load	Sample	20	5
Wash	A	100	5
Elute	B	100	5
Flush	Air	100	20

Solvent A: Water
Solvent B: Methanol

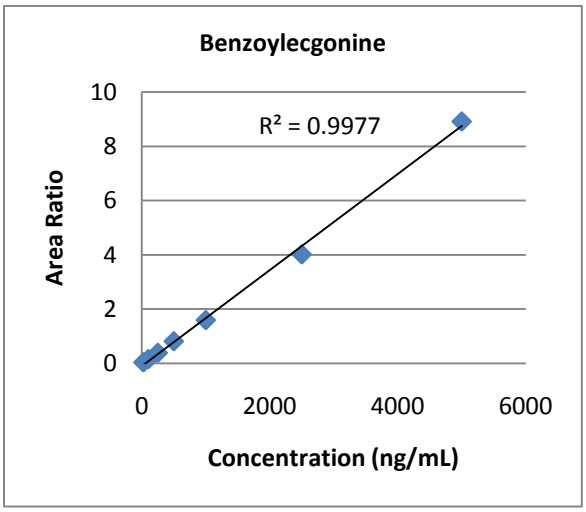
Samples were analyzed in-line with the following method:

Analysis Method

Instrument:	Finnigan TSQ Quantum Ultra AM triple quadrupole with Agilent 1200 Rapid Resolution HPLC
Column:	Agilent XDB-C18, 2.1x50mm, 3.5 µm particles
Solvent A:	Water with 0.1% Formic acid
Solvent B:	Methanol with 0.1% Formic acid
Column Temp.:	Ambient
Flow Rate:	0.6 mL/min
Gradient:	0.00 min (80% A), 0.5 min (80%A), 4.0 min (0% A), 5.5 min (0% A), 6.0 min (80%A)
Ionization Mode:	Positive Ion (HESI) Heated Electrospray Ionization Probe
SRM Channels:	BZE m/z 290-168, BZE internal standard m/z 293-171

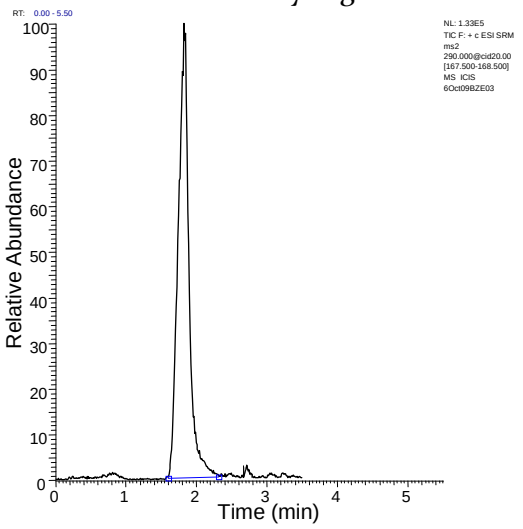
Results

Standards of BZE were prepared in urine over the range of 25 - 5000 ng/mL and processed in-line with the ITSP cleanup procedure followed by LC/MS/MS. The calibration curve that was obtained is shown below, plotting the analyte to internal standard area ratio versus the concentration of the analyte.

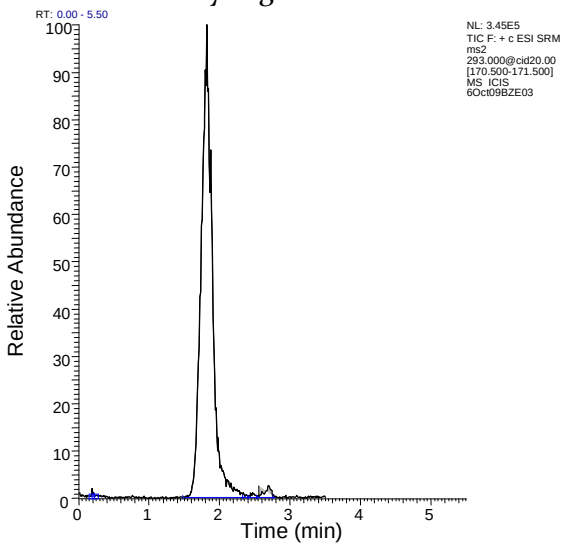


Example chromatograms of the SRM channels for BZE (m/z 290-168) and its internal standard (m/z 293-171) for the 250 ng/mL standard in urine with ITSP cleanup are shown below.

Benzoylecgonine



Benzoylecgonine Internal Standard



Conclusions

A simplified, automated procedure for the analysis of benzoylecgonine in human urine has been developed using reversed phase (C1) ITSP solid phase extraction (SPE) for in-line sample cleanup and LC/MS/MS for detection. A linear response was observed over the concentration range of 25 - 5000 ng/mL.

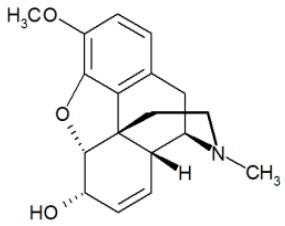
[1]Mandatory Guidelines for Federal Workplace Drug Testing Programs, Federal Register notice published April 13, 2004 (69 FR 19644) effective Nov. 1, 2004.

Determination of Total Codeine, Oxycodone, Hydrocodone, Morphine, Oxymorphone, and Hydromorphone in Human Urine Using Automated, In-Line, ITSP Solid Phase Extraction and Liquid Chromatography Mass Spectrometric Detection

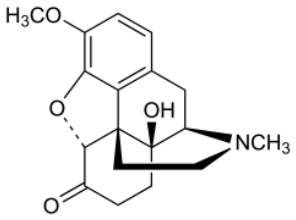
Thurman L. Allsup[1], Kenneth C. Lewis[1] and Kim Gamble[2]
[1]OpAns, LLC, Durham NC, 27713, [2]Microliter Analytical Supplies, Inc., Suwannee GA, 30024

Abstract

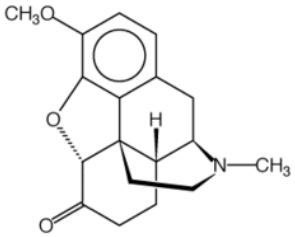
A quantitative analytical method has been developed for the determination of total (free and conjugated) codeine, oxycodone, hydrocodone, morphine, oxymorphone, and hydromorphone in human urine after acid hydrolysis. Opiates are one of the main drug classes analyzed by forensic and clinical labs. Historical analytical procedures use acid hydrolysis of the urine to cleave the glucuronide conjugates followed by the addition of reagents to prepare the hydrolysate for extraction by SPE. Generally the extracted sample eluant is evaporated to dryness in preparation for derivatization to allow analysis by GC/MS. This method is extremely labor intensive. The method that has been developed here is fully automated to quantitatively extract total opiates from urine using strong cation exchange ITSP SPE devices and analyze the samples in-line by LC/MS/MS. The sample extraction and injection onto the LC/MS/MS were both performed in-line by a PAL (CTC) sample handling unit. Spiked urine samples with stable label internal standards were hydrolyzed with hydrochloric acid and without further preparation applied to strong cation exchange ITSP devices for extraction. The extracts were injected in-line onto an LC/MS/MS using a XDB-C18 (Agilent) column. The extracted standard curve had a range of 100-5000 ng/mL for morphine, codeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone. All six compounds had correlation coefficients greater than 0.995. Selected reaction monitoring (SRM) was used for quantitation. An additional SRM transition was acquired for each compound. The ion ratio of the quantitative transition to the confirmatory transition was used in order to confirm the presence of the drug.



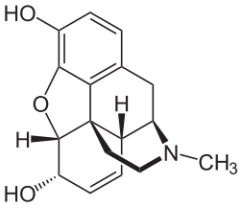
Codeine
Exact Mass 299.4



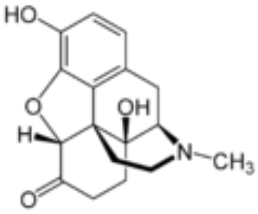
Oxycodone
Exact Mass 315.4



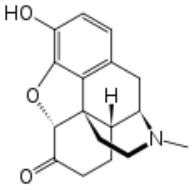
Hydrocodone
Exact Mass 299.4



Morphine
Exact Mass 285.3



Oxymorphone
Exact Mass 301.3



Hydromorphone
Exact Mass 285.3

Experimental

Sample Preparation

All opiate standards and their deuterated internal standards were obtained from Cerilliant. Standards of the opiates were prepared in urine at nominal concentrations of 100, 250, 500, 1000, 2500, and 5000 ng/mL. Stable label internal standards (250 ng/mL of each) were added to the urine. Hydrochloric acid (100 µL) was added to each 1 mL of urine sample and heated at 100°C for 30 minutes.

ITSP SPE Method

ITSP Cartridges: Jordi Strong Cation Exchange 10mg (Product No.: 07-JSCX10-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

Step	Solvent	Volume (µL)	Flowrate (µL/sec)
Condition	B	100	15
Condition	A	100	15
Condition	C	100	15
Load	Sample	50	5
Wash	A	100	15
Wash	D	100	15
Wash	B	100	15
Elute	E	100	5
Flush	Air	100	50

Solvent A: Water
Solvent B: Methanol
Solvent C: Phosphate buffer pH 6
Solvent D: Acetate buffer pH 4.5
Solvent E: Water:Acetonitrile:Ammonium Hydroxide (7:3:0.2)

Samples were analyzed in-line with the following method:

Analysis Method

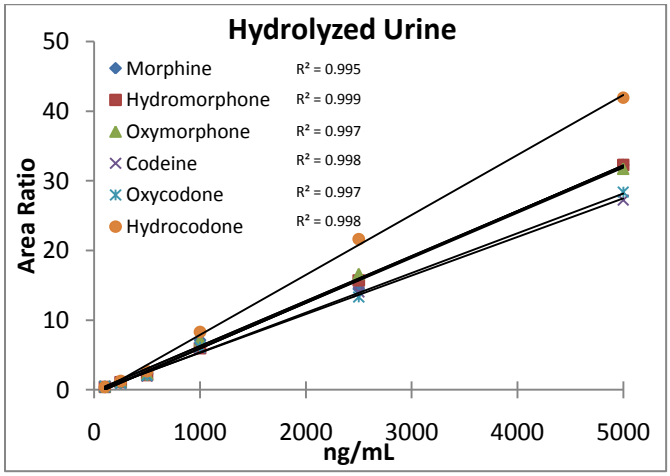
Instrument: Finnigan TSQ Quantum Ultra AM triple quadrupole with Agilent 1200 Rapid Resolution HPLC
Column: Agilent XDB-C18, 2.1x50mm, 3.5 µm particles
Solvent A: Water with 0.1% Formic acid
Solvent B: Methanol with 0.1% Formic acid
Column Temp.: Ambient
Flow Rate: 0.4 mL/min
Gradient: 0.00 min (100% A), 0.5 min (100%A), 6.0 min (20% A), 6.2 min (100% A),
Ionization Mode: Positive Ion (HESI) Heated Electrospray Ionization Probe

SRM Channels:

Drug	Quant.	Confirm	IS
Morphine	286-152	286-165	289-152
Hydromorphone	286-185	286-157	289-185
Oxymorphone	302-284	302-227	305-287
Codeine	300-165	300-152	303-165
Hydrocodone	300-199	300-128	303-199
Oxycodone	316-241	316-256	319-244

Results

Standards of the opiates were prepared in urine over the range of 100-5000 ng/mL, internal standard was added, samples were hydrolyzed and then processed with the ITSP cleanup procedure prior to analysis by LC/MS/MS. The calibration curves that were obtained are shown below, plotting the analyte to internal standard area ratio versus the concentration.



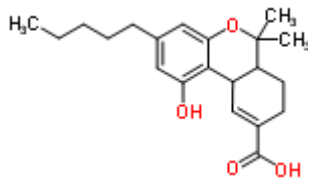
The ratio of the confirmatory transition to the quantitation transition was calculated for each drug at each concentration. The mean confirmatory ratios for morphine, hydromorphone, oxymorphone, codeine, hydrocodone, and oxycodone were 1.26, 1.60, 2.65, 0.93, 2.90, and 1.35, respectively. The confirmatory ratios for all standards were found to be within 15% of the mean for that drug.

Determination of 11-Nor-Δ9-Tetrahydrocannabinol-9-carboxylic acid in Human Urine Using Automated, In-Line, ITSP Solid Phase Extraction and Liquid Chromatography Mass Spectrometric Detection

Thurman L. Allsup[1], Kenneth C. Lewis[1] and Kim Gamble[2]
[1]OpAns, LLC, Durham NC, 27713, [2]Microliter Analytical Supplies, Inc., Suwannee GA, 30024

Abstract

A quantitative analytical method has been developed for the determination of 11-nor-Δ9-tetrahydro-cannabinol-9-carboxylic acid (THCA) in human urine. THCA is the primary metabolite of Δ9-tetrahydrocannabinol found in urine and is the species used to confirm marijuana use. Historical analytical procedures for the analysis of THCA in urine involve the extraction of THCA from the urine matrix followed by the evaporation of the eluant solvent and a multi-stepped derivatization procedure to prepare the sample for GC/MS. The method that has been developed here is fully automated to quantitatively extract THCA from urine using C18 ITSP SPE devices and analyze the samples in-line by LC/MS/MS. The sample extraction and injection onto the LC/MS/MS were both performed in-line by a PAL (CTC) sample handling unit. Spiked urine samples with stable label internal standards were applied to C18 ITSP devices for extraction. The extracts were injected in-line onto an LC/MS/MS using a XDB-C18 (Agilent) column for separation. The extracted standard curve had a range of 100 – 2500 ng/mL for THCA. The correlation coefficient was greater than 0.998. Selected reaction monitoring (SRM) was used for the quantitation. An additional SRM transition was acquired and the ion ratio of the quantitative transition to the confirmatory transition was used in order to confirm the presence of the drug.



THCA
Exact Mass 344.2

Experimental

Sample Preparation

11-Nor-Δ9-tetrahydrocannabinol-9-carboxylic acid standard and its deuterated analog were obtained from Cerilliant. A set of standards was prepared in human urine. Calibration standards were prepared at nominal concentrations of 100, 250, 500, 1000, and 2500 ng/mL with a constant concentration of the deuterated analog at 500 ng/mL.

ITSP SPE Method

ITSP Cartridges: SPE µLplate w/C18 10mg (Product No.: 07-C1810-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

Step	Solvent	Volume (µL)	Flow Rate (µL/sec)
Condition	B	100	10
Condition	A	100	10
Load	Sample	20	5
Wash	A	100	10
Elute	C	100	2
Flush	Air	100	50

Solvent A: Water
Solvent B: Methanol
Solvent C: Isopropanol

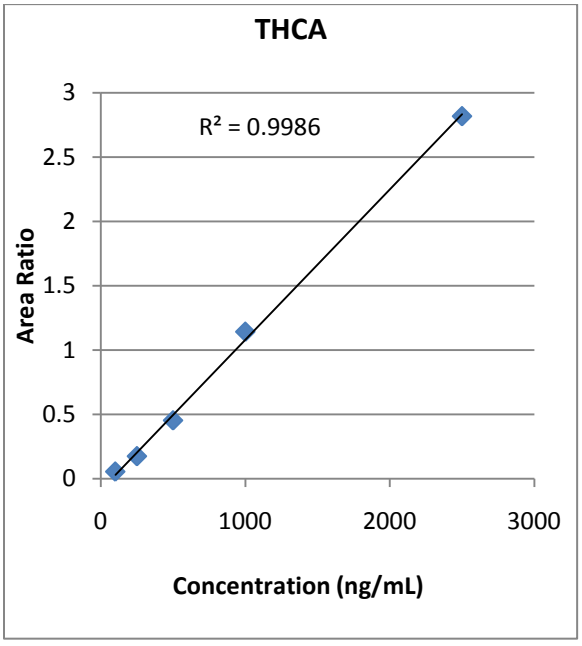
Samples were analyzed in-line with the following method:

Analysis Method

Instrument:	Finnigan TSQ Quantum Ultra AM triple quadrupole with Agilent 1200 Rapid Resolution HPLC
Column:	Agilent XDB-C18, 2.1x50mm, 3.5 µm particles
Solvent A:	Water with 0.1% Formic acid
Solvent B:	Methanol with 0.1% Formic acid
Column Temp.:	Ambient
Flow Rate:	0.6 mL/min
Gradient:	0.00 min (60% A), 0.5 min (60%A), 4.0 min (0% A), 5.5 min (0% A), 6.0 min (60%A),
Ionization Mode:	Positive Ion (HESI) Heated Electrospray Ionization Probe
SRM Channels:	THCA Quantitative m/z 345-327, THCA Confirmatory m/z 345-299, THCA Internal Standard m/z 348-330

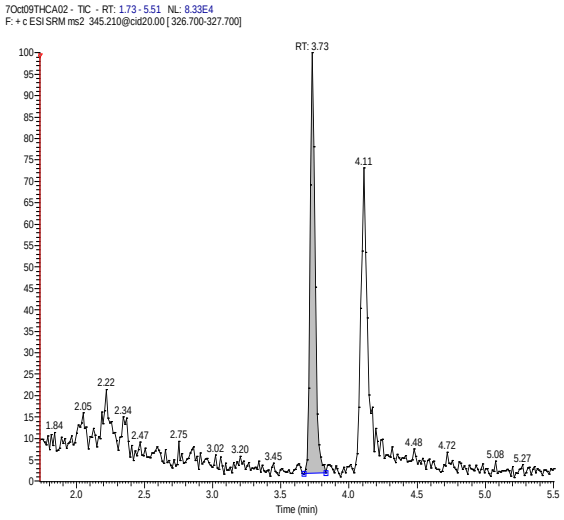
Results

Standards of THCA were prepared in urine over the range of 100 - 2500 ng/mL and processed in-line with the ITSP cleanup procedure followed by LC/MS/MS. The calibration curve that was obtained is shown below, plotting the analyte to internal standard area ratio versus the concentration of the analyte.



The lower limit of quantitation for THCA in urine is 100 ng/mL. An example chromatogram of the SRM channel for THCA (m/z 345-327) for the 100 ng/mL standard extracted from urine with ITSP cleanup is shown below.

11-Nor-Δ9-Tetrahydrocannabinol-9-carboxylic acid



The ratio of the confirmatory transition to the quantitation transition was calculated for THCA at each concentration. The mean confirmatory ratio for THCA was 1.22. The confirmatory ratios for all standards were found to be within 5% of the mean.

Conclusions

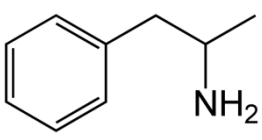
A simplified, automated procedure for the analysis of 11-Nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in human urine has been developed using reversed phase (C18) ITSP solid phase extraction (SPE) for in-line sample cleanup and LC/MS/MS for detection. A linear response was observed over the concentration range of 100 - 2500 ng/mL.

Determination of Amphetamine, Methamphetamine and Phencyclidine in Human Urine Using Automated, In-Line, ITSP Solid Phase Extraction and Liquid Chromatography Mass Spectrometric Detection

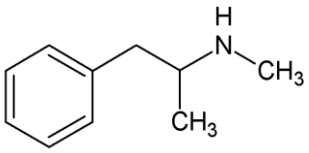
Thurman L. Allsup[1], Kenneth C. Lewis[1] and Kim Gamble[2]
[1]OpAns, LLC, Durham NC, 27713, [2]Microliter Analytical Supplies, Inc., Suwannee GA, 30024

Abstract

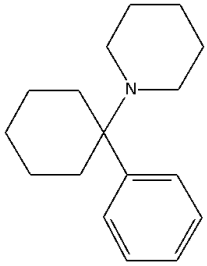
A quantitative analytical method has been developed for the determination of amphetamine, methamphetamine and phencyclidine (PCP) in human urine. Amphetamines and PCP are two of the main drug classes analyzed by forensic and clinical labs. Historical analytical procedures involve the extraction of the drugs by SPE followed by the evaporation of the eluant in preparation for derivatization to allow analysis by GC/MS. The method presented here is fully automated to quantitatively extract amphetamines and PCP from urine using strong cation exchange ITSP SPE devices and analyze the samples in-line by LC/MS/MS. The sample extraction and injection onto the LC/MS/MS were both performed in-line by a PAL (CTC) sample handling unit. Spiked urine samples with stable label internal standards were applied to strong cation exchange ITSP devices for extraction. The extracts were injected in-line onto an LC/MS/MS using a XDB-C18 (Agilent) column for separation. The extracted standard curve had a range of 25 - 5000 ng/mL for amphetamine, methamphetamine and phencyclidine. All three compounds had correlation coefficients greater than 0.999. Selected reaction monitoring (SRM) was used for the quantitation. An additional SRM transition was acquired for each compound. The ion ratio of the quantitative transition to the confirmatory transition was used in order to confirm the presence of the drug.



Amphetamine
Exact Mass 135.2



Methamphetamine
Exact Mass 149.2



Phencyclidine
Exact Mass 243.4

Experimental

Sample Preparation

Amphetamine, methamphetamine and phencyclidine standards and their deuterated internal standards were obtained from Cerilliant. Standards were prepared in urine at nominal concentrations of 25, 100, 250, 500, 1000, 2500, and 5000 ng/mL. Stable label internal standards (500 ng/mL of each) were added to the urine standards.

ITSP SPE Method

ITSP Cartridges: Jordi Strong Cation Exchange 10mg (Product No.: 07-JSCX10-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

Step	Solvent	Volume (µL)	Flowrate (µL/sec)
Condition	B	100	30
Condition	A	100	30
Condition	C	100	30
Load	Sample	50	5
Wash	A	100	20
Wash	D	100	20
Wash	B	100	20
Elute	E	100	5
Flush	Air	100	20

Solvent A: Water
Solvent B: Methanol
Solvent C: Phosphate buffer pH 6
Solvent D: Acetate buffer pH 4.5
Solvent E: Methanol:Isopropanol:Ammonium Hydroxide (78:20:2)

Samples were analyzed in-line with the following method:

Analysis Method

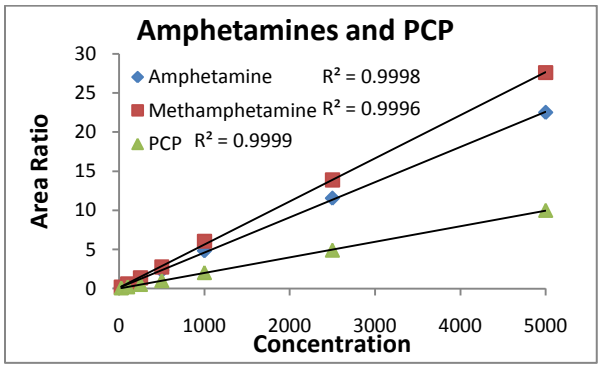
Instrument: Finnigan TSQ Quantum Ultra AM triple quadrupole with Agilent 1200SL HPLC
Column: Agilent XDB-C18, 2.1x50mm, 3.5 µm particles
Solvent A: Water with 0.1% Formic acid
Solvent B: Methanol with 0.1% Formic acid
Column Temp.: Ambient
Flow Rate: 0.6 mL/min
Gradient: 0.0 min (100% A), 1 min (100%A), 4.0 min (50% A), 4.5 min (50% A), 5.5 min (100% A)
Ionization Mode: Positive Ion (HESI) Heated Electrospray Ionization Probe

SRM Channels:

	Quant.	Confirm	IS
Amphetamine	136-92	136-119	144-98
Methamphetamine	150-92	150-120	158-94
Phencyclidine	244-92	244-160	249-164

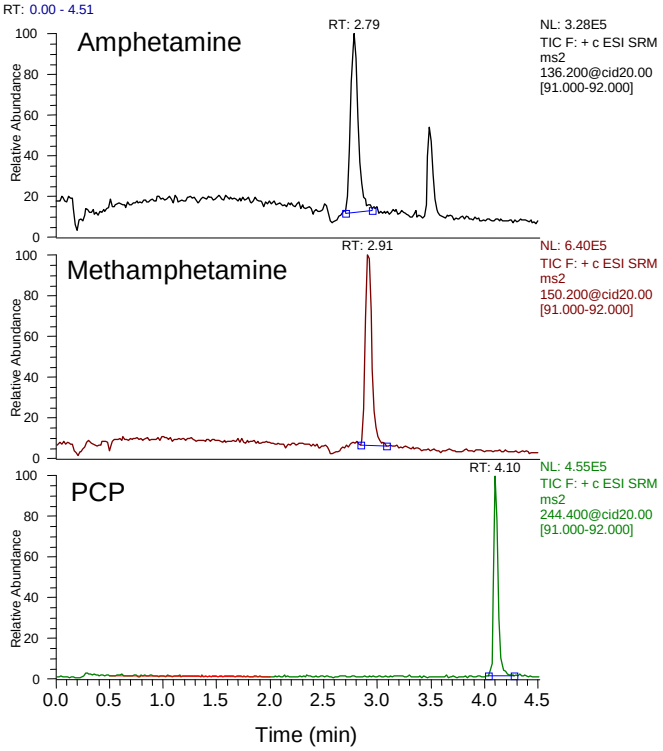
Results

Standards of the amphetamines and PCP were prepared in urine over the range of 25-5000 ng/mL, internal standard was added, samples were processed with the ITSP cleanup procedure. The calibration curves that were obtained for each are shown below, plotting the analyte to internal standard area ratio versus the concentration. The correlation coefficient was greater than 0.999 in all cases.



The ratio of the confirmatory transition to the quantitation transition was calculated for each drug at each concentration. The mean confirmatory ratios for amphetamine, methamphetamine, and PCP were 1.5, 3.2, and 1.1, respectively. The confirmatory ratios for all standards were found to be within 5% of their mean.

Chromatograms of the extracted 25 ng/mL standard of amphetamine, methamphetamine and PCP in urine are shown below.



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ITSP Components by MicroLiter:

Part Number	Description
• 07-ITSP-HW	Hardware kit to modify CTC PAL for use with ITSP
• 07-C1810-20A	ITSP SPE device – 10mg Orochem C18 Reverse Phase
• 07-JSCX10-20A	ITSP SPE device – 10mg Jordi Strong Cation Exchange

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