Determination of Bath Salts (Pyrovalerone Analogs) in Biological Samples

Application Note

Forensic Toxicology

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Abstract

A method has been developed on the Agilent 220 Quadrupole Ion Trap using EI-MS/MS for the identification and quantification of Pyrovalerone Analogs in biological samples. A working range of 50–1,000 µg/mL shows the method linearity of the Pyrovalerone Analogs

Introduction

Pyrrolidinopentiphenone (PVP), Methylenedioxypyrovalerone (MDPV) and Napthylpyrovalerone (Naphyrone) are designer drugs marketed as bath salts. These drugs are analogs of Pyrovalerone. The drugs are marketed as legal substitutes for drugs such as Cocaine and MDMA.

This application note describes a method for the analysis of serum whole blood, vitreous fluid, urine, or tissue homogenate specimens. A minimum of 3 mL of sample is required for analysis.

Pyrrolidinopentiphenone (PVP), Pyrovalerone, MDPV, and Naphyrone and the internal standard Ropivacaine are extracted from alkalinized samples into an organic solvent using a liquid-liquid method of extraction. The extracts are reconstituted with ethyl acetate and injected into the Quadrupole Ion Trap GC/MS/MS for analysis.

Agilent Technologies

Experimental

Standards and reagents

Reagents

- N-Chlorobutane (Nanograde), ethyl acetate, concentrated ammonia (NH_4OH), sodium carbonate/bicarbonate buffer pH = 9.8 (mix 100 g Na_2CO_3 and 50 g $NaHCO_3$ in 1,000 mL de-ionized water, adjust the pH to 9.8). Stable for 1 year at room temperature.
- Concentrated HCl, methanol (HPLC Grade), 0.1% HCl in methanol (dilute 0.1 mL of concentrated HCl in 100 mL of methanol. Stable for 1 year at room temperature).
- MDPV, naphyrone, and pyrovalerone stock standards (1 mg/mL in methanol) were purchased from Cerilliant (Store in freezer until outdated).
- PVP stock standard purchased from Cayman Chemical (10-mg bottle), transfer contents to a 10-mL flask and fill to mark with methanol. Stable for 2 years in a freezer.
- Ropivacaine stock standard purchased from Sigma (R0283), 11.9 mg diluted to 10 mL with methanol. Stable for 2 years in a freezer.
- MDPV QC stock standard purchased from Cayman Chemical, 5 mg in 50 µL of Methyl Acetate (dilute to 5 mL in methanol). Stable for 1 year stored in a freezer.
- Naphyrone QC stock standard (1 mg/mL in methanol) purchased from Cerilliant. Store in a freezer until outdated.
- Pyrovalerone QC stock standard purchased from Cayman Chemical (5 mg in 100 µL of methanol), transfer contents to a 5-mL flask and dilute to mark with methanol. Stable for 2 years in freezer.
- PVP QC stock standard was purchased from Cayman Chemical as 10-mg bottle (transfer contents to a 10-mL flask and dilute to mark with methanol. Stable for 2 years in a freezer.

Working standards

- MDPV, naphyrone, pyrovalerone, PVP calibration intermediate standard 15 µg/mL (add 150 µL of each stock standard to a 10-mL flask and fill to 10 mL with methanol).
- MDPV, naphyrone, pyrovalerone, PVP QC intermediate standard 15 µg/mL (add 150 µL of each stock standard to a 10-mL flask and fill to 10 mL with methanol).
- Working internal standard ropivacaine 15 µg/mL (add 150 µL of stock solution to a 10-mL flask and fill to 10 mL with methanol). Store at 2–8 °C. Stable for 1 year.

Controls and Calibration Standards

Negative Control- drug free whole blood obtained from American Red Cross or pooled blank urine. The matrix must be tested prior to use and found to be drug free. When stored at –20 °C, it is stable for 1 year.

Low Control (125 ng/mL) - Prepared in blood or urine fresh as needed, 25 µL of the QC Intermediate Standard to 3 mL blank blood or urine.

High Control (500 ng/mL) - Prepared in blood or urine fresh as needed, 100 µL of the QC Intermediate Standard to 3 mL blank blood or urine.

Sample Preparation

Prepare a calibration curve using the working standard and **drug free blood or urine** as follows:

50 ng/mL - 10 µL std. and 3 mL blood/urine,
100 ng/mL - 20 µL std. and 3 mL blood/urine,
250 ng/mL - 50 µL std. and 3 mL blood/urine,
500 ng/mL - 100 µL std. and 3 mL blood/urine
1,000 ng/mL - 200 µL std. and 3 mL blood/urine

Pipet 3 mL of samples, negative, and positive controls into labeled 16 × 100 mm culture tubes, Add 50 µL of working internal standard and 2 mL of pH 9.8 Carbonate Buffer, add two drops of NH_4OH to each tube and vortex gently to mix. Add 7.0 mL of *n*-butyl chloride to each tube, cap, and rotate all tubes for at least 10 minutes. Centrifuge all tubes at 3,000 RPM for 10 minutes. Transfer organic (top) layer to a clean, labeled 16 × 100 culture tube. Add two drops of 0.1% methanolic HCl and evaporate to dryness at 37 °C with nitrogen. Reconstitute dried extracts with 200 µL of ethyl acetate and transfer to autosampler vials with inserts, cap and transfer to the GC/MS/MS for analysis.

GC/MS Ion Trap Analysis

Column	DB-5MS or equivalent 25 m × 200 mm, 0.33 µm
Injection volume	0.5 µL
Injection mode	Splitless
Inlet temperature	250 °C
Carrier gas	Helium
Column flow	1.3 mL/min
Oven program	70 °C; 1 minute hold 25 °C/min to 310 °C; 4.4 minute hold

Quadrupole Ion Trap MS Conditions

Tune	Auto-tune
Acquisition	EI-MS/MS 50–200 da
Solvent delay	7.0 minutes
MS temperatures	Trap 210 °C, Manifold 50 °C, Transfer line 310 °C

Compound	Rt(min)	Precursor	Quant ion	Qualifiers	Excit volt	Filament	Multiplier	Target
PVP	8.1	126	84	124/97	0.5 V	50 µA	+50 V	3,000
Provalerone	8.6	126	84	124/97	0.5 V	50 µA	+50 V	3,000
MDPV	9.68	126	84	124/97	0.5 V	50 µA	+50 V	3,000
Ropivacaine IS	9.95	126	84	98/56	0.5 V	50 µA	+50 V	3,000
Naphyrone	10.38	126	84	124/97	0.5 V	50 µA	+50 V	3,000

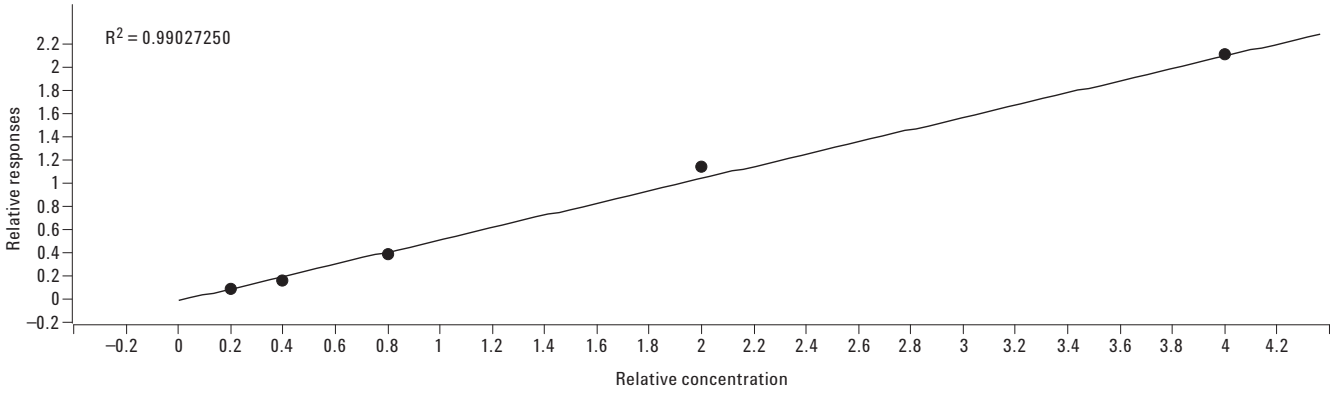
Results and Discussion

The following criteria are used to determine the presence and amount of the pyrovalerone analog:

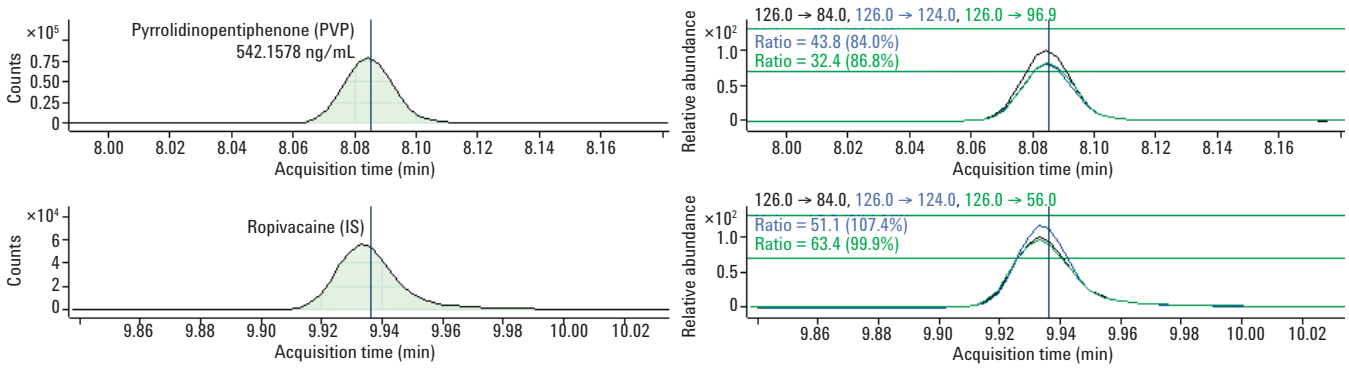
- The chromatography is acceptable (peak resolution, peak symmetry, absence of carryover). The selected ions for quantitation and qualification are present. The retention times of the presumed pyrovalerone analog from the test specimen is within ± 2% of the retention times for the latest calibration.
- The area of the analog and the internal standard quantitative ions are used for quantitative analysis. Quantitation is accomplished by comparison of the relative response of unknowns and controls against a calibration curve produced from the relative responses for each calibrator concentration. The positive controls must be within their target ranges and the pyrovalerone analogs must be absent in the negative control.
- Appropriate *m/z* ions must be observed. The test specimens and positive controls must exhibit *m/z* ions resulting from the ionization of 126 *m/z* at the respective retention times for pyrovalerone, MDPV, PVP, naphyrone, and ropivacaine IS.
- The two ion ratio method is used for identification. For Pyrovalerone, PVP, MDPV, and Naphyrone the abundance for the *m/z* ion peaks 124 and 97 are divided by the ion abundance of base ion peak 84. Calculated ion ratios are within 20% of the target values determined from the calibration.

Linearity	50–1,000 ng/mL
Limit of detection (LOD)	20 ng/mL
Limit of quantitation (LOQ)	50 ng/mL
Interferences	None were noted

PVP Calibration



Method Limits

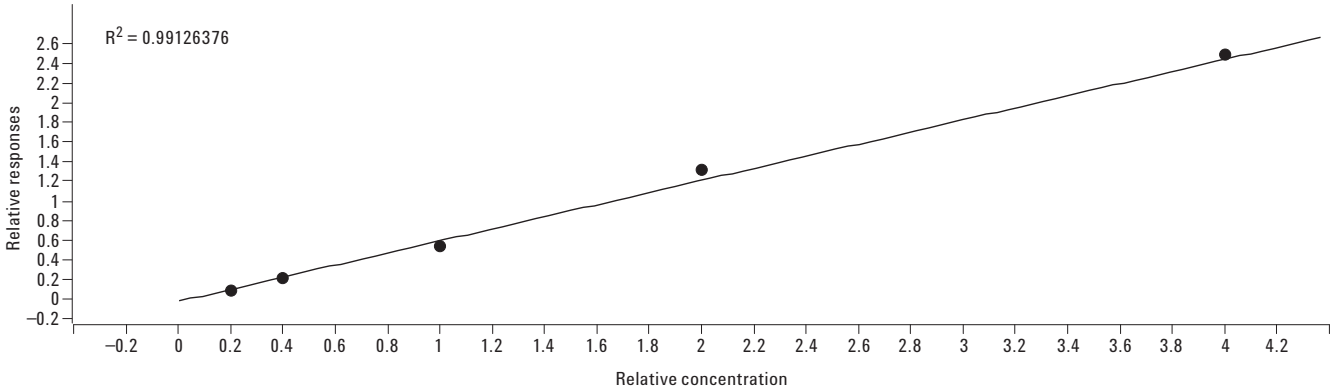


Batch Results

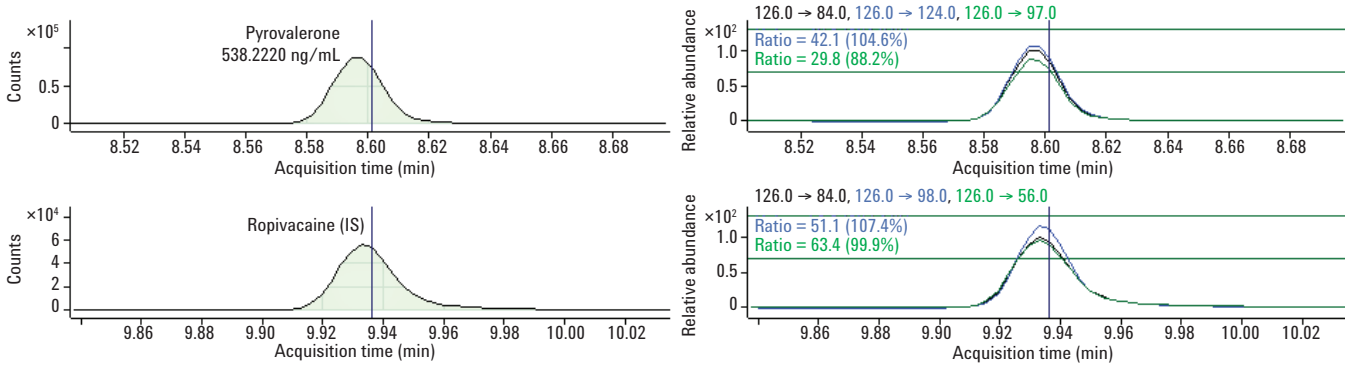
		Sample					Pyrrolidin...	Pyrrolidinopentiphenone(PVP) Results							Qualifier...		Qualifier...		Ropivacaine(I.S.) (ISTD...		Qualifier...		Qualifier...	
ⓘ		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		CAL1	CAL1 3-5-2012 3-12-22 PM.SMS.D	Cal	1	3/5/2012 1:12 PM	50.0000	8.086	7373		39.8945	39.8945	79.8	48.5		35.8		9.935	87567	53.7		60.2	
		CAL2	CAL2 3-5-2012 3-34-07 PM.SMS.D	Cal	2	3/5/2012 1:34 PM	100.0000	8.083	12688		76.8121	76.8121	76.8	52.1		35.6		9.934	78265	52.1		57.5	
		CAL3	CAL3 3-5-2012 3-55-54 PM.SMS.D	Cal	3	3/5/2012 1:55 PM	200.0000	8.084	32611		183.7979	183.7979	91.9	48.4		32.7		9.931	84069	50.9		59.8	
		CAL4	CAL4 3-5-2012 4-17-44 PM.SMS.D	Cal	4	3/5/2012 2:17 PM	500.0000	8.084	77431		544.7069	544.7069	108.9	44.1		32.1		9.935	67355	54.6		64.6	
		CAL5	CAL5 3-5-2012 4-39-40 PM.SMS.D	Cal	5	3/5/2012 2:39 PM	1000.00...	8.084	193270		1004.7886	1004.7886	100.5	47.0		27.2		9.932	91140	55.1		60.0	
!		NEG	NEG 3-5-2012 5-01-23 PM.SMS.D	Sample		3/5/2012 3:01 PM												9.933	77787	51.2		62.3	
		LOW	LOW 3-5-2012 5-23-10 PM.SMS.D	Sample		3/5/2012 3:23 PM		8.085	19223		115.5457	115.5457		47.8		36.3		9.931	78828	52.0		60.7	
		HIGH	HIGH 3-5-2012 5-44-59 PM.SMS.D	Sample		3/5/2012 3:45 PM		8.085	92200		542.1578	542.1578		43.8		32.4		9.933	80580	51.1		63.4	
!		BLK	BLK 3-5-2012 9-01-38 PM.SMS.D	Sample		3/5/2012 7:01 PM																	
!		208 BLOOD	208 BLOOD 3-5-2012 9-23-28 PM.SMS.D	Sample		3/5/2012 7:23 PM												9.932	69336	55.1		64.9	
!	▼	1027 BLOOD	1027 BLOOD 3-5-2012 9-45-10 PM.SMS.D	Sample		3/5/2012 7:45 PM		8.085	2074		14.6005	14.6005		54.9		34.9		9.934	67319	52.0		61.0	
!		1027 URINE	1027 URINE 3-5-2012 10-07-10 PM.SMS.D	Sample		3/5/2012 8:07 PM		8.085	15466		81.4251	81.4251		47.0		34.2		9.933	89998	56.1		58.9	

Note tags for outliers and below calibration.

Pyrovalerone Calibration



500 ng/mL Standard

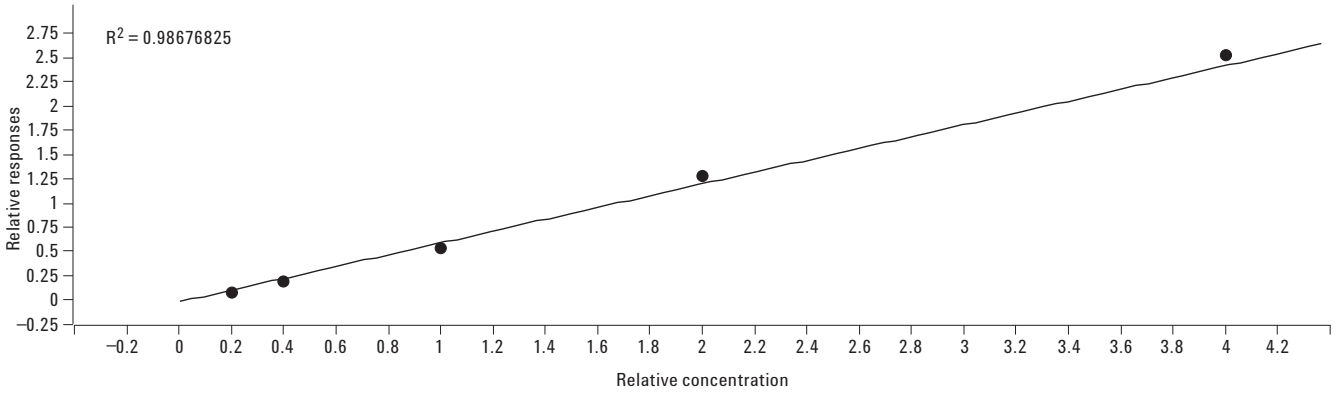


Batch Results

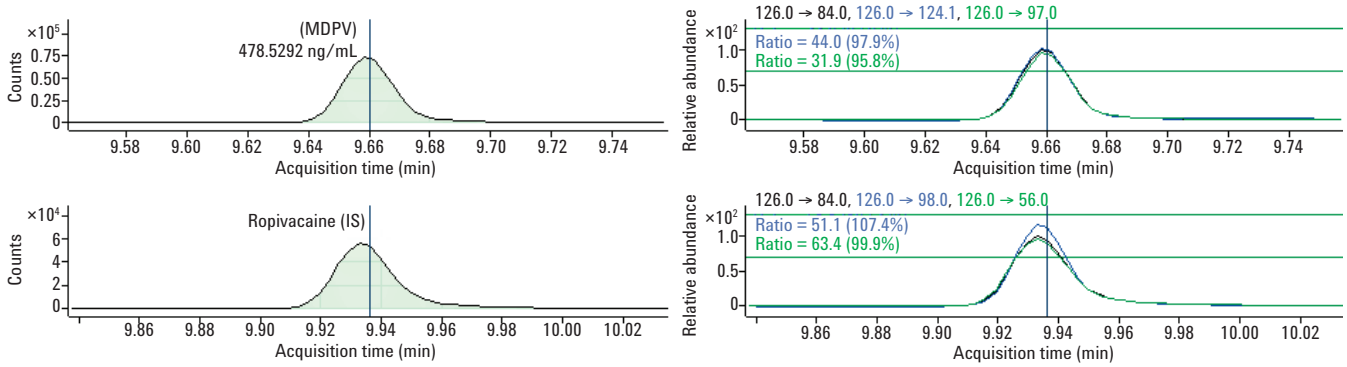
Sample							Pyrovaler...	Pyrovalerone Results							Qualifier...		Qualifier...		Ropivacaine(...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		CAL1	CAL1 3-5-2012 3-12-22 PM.SMS.D	Cal	1	3/5/2012 1:12 PM	50.0000	8.598	8074		37.5523	37.5523	75.1	48.2		34.0		9.935	87567	53.7		60.2	
		CAL2	CAL2 3-5-2012 3-34-07 PM.SMS.D	Cal	2	3/5/2012 1:34 PM	100.0000	8.595	16529		86.0177	86.0177	86.0	48.4		37.2		9.934	78265	52.1		57.5	
		CAL3	CAL3 3-5-2012 3-55-54 PM.SMS.D	Cal	3	3/5/2012 1:55 PM	250.0000	8.594	45618		221.0053	221.0053	88.4	48.2		35.5		9.931	84069	50.9		59.8	
		CAL4	CAL4 3-5-2012 4-17-44 PM.SMS.D	Cal	4	3/5/2012 2:17 PM	500.0000	8.596	89135		538.9924	538.9924	107.8	45.2		29.2		9.935	67355	54.6		64.6	
		CAL5	CAL5 3-5-2012 4-39-40 PM.SMS.D	Cal	5	3/5/2012 2:39 PM	1000.0000	8.599	227447		1016.4323	1016.4323	101.6	45.0		27.0		9.932	91140	55.1		60.0	
		NEG	NEG 3-5-2012 5-01-23 PM.SMS.D	Sample		3/5/2012 3:01 PM												9.933	77787	51.2		62.3	
		LOW	LOW 3-5-2012 5-23-10 PM.SMS.D	Sample		3/5/2012 3:23 PM		8.596	23061		119.1517	119.1517		44.6		32.7		9.931	78828	52.0		60.7	
		HIGH	HIGH 3-5-2012 5-44-59 PM.SMS.D	Sample		3/5/2012 3:45 PM		8.595	106484		538.2220	538.2220		42.1		29.8		9.933	80580	51.1		63.4	
		BLK	BLK 3-5-2012 9-01-38 PM.SMS.D	Sample		3/5/2012 7:01 PM																	
		208 BLOOD	208 BLOOD 3-5-2012 9-23-28 PM.SMS.D	Sample		3/5/2012 7:23 PM												9.932	69336	55.1		64.9	
		1027 BLOOD	1027 BLOOD 3-5-2012 9-45-10 PM.SMS.D	Sample		3/5/2012 7:45 PM												9.934	67319	52.0		61.0	
		1027 URINE	1027 URINE 3-5-2012 10-07-10 PM.SMS.D	Sample		3/5/2012 8:07 PM												9.933	89998	56.1		58.9	

Note tags for outliers and below calibration.

MDPV Calibration



500 ng/mL Standard

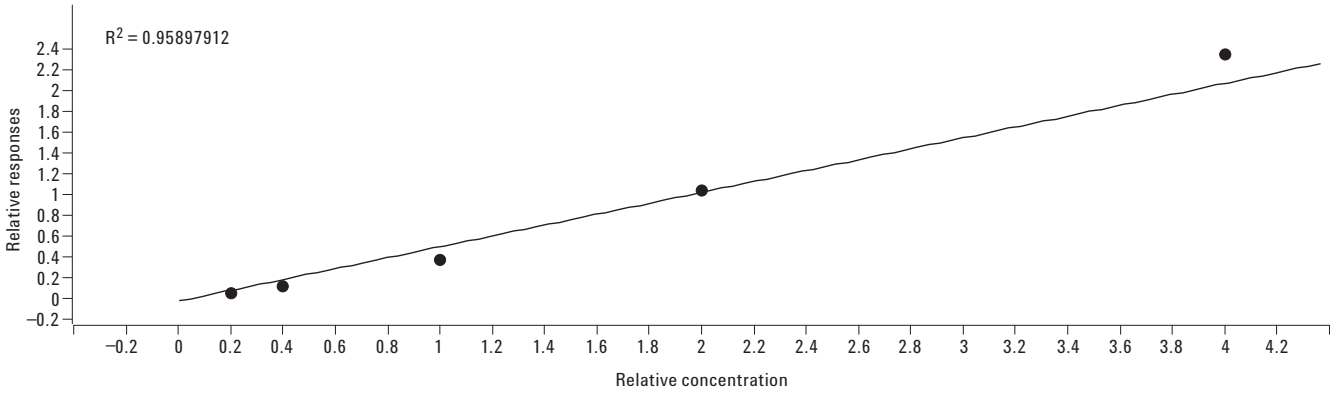


Batch Results

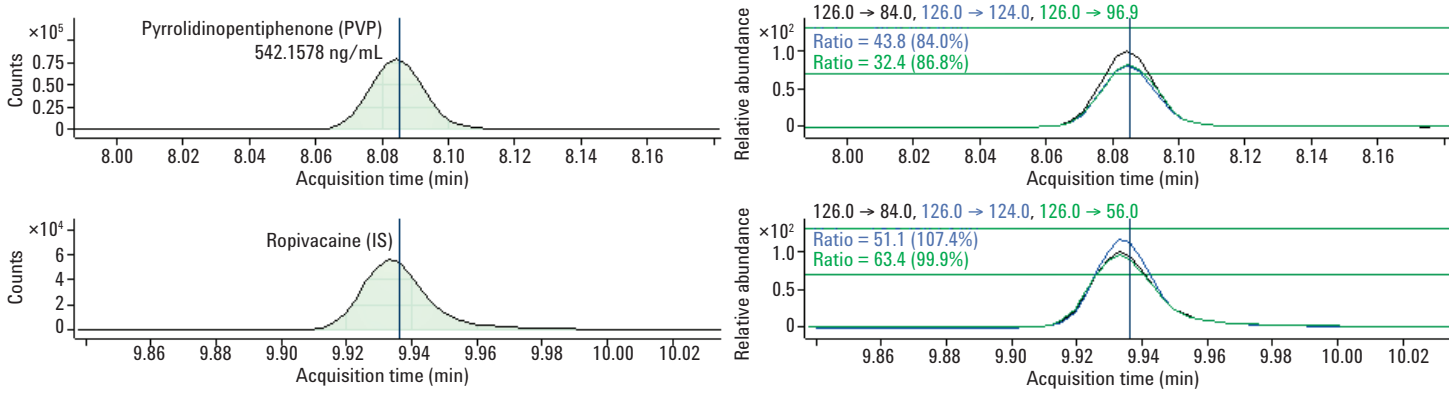
Sample							MDPV Me...	MDPV Results							Qualifier...		Qualifier...		Ropivacaine(...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		CAL1	CAL1 3-5-2012 3-12-22 PM.SMS.D	Cal	1	3/5/2012 1:12 PM	50.0000	9.663	6576		30.8638	30.8638	61.7	49.6		37.5		9.935	87567	53.7		60.2	
		CAL2	CAL2 3-5-2012 3-34-07 PM.SMS.D	Cal	2	3/5/2012 1:34 PM	100.0000	9.659	15315		80.4290	80.4290	80.4	43.3		34.9		9.934	78265	52.1		57.5	
		CAL3	CAL3 3-5-2012 3-55-54 PM.SMS.D	Cal	3	3/5/2012 1:55 PM	250.0000	9.660	44714		218.6051	218.6051	87.4	45.2		34.7		9.931	84069	50.9		59.8	
		CAL4	CAL4 3-5-2012 4-17-44 PM.SMS.D	Cal	4	3/5/2012 2:17 PM	500.0000	9.660	86729		529.2326	529.2326	105.8	45.3		33.3		9.935	67355	54.6		64.6	
		CAL5	CAL5 3-5-2012 4-39-40 PM.SMS.D	Cal	5	3/5/2012 2:39 PM	1000.0000	9.662	230809		1040.8694	1040.8694	104.1	43.3		27.9		9.932	91140	55.1		60.0	
		NEG	NEG 3-5-2012 5-01-23 PM.SMS.D	Sample		3/5/2012 3:01 PM												9.933	77787	51.2		62.3	
		LOW	LOW 3-5-2012 5-23-10 PM.SMS.D	Sample		3/5/2012 3:23 PM		9.661	19252		100.3791	100.3791		43.8		33.9		9.931	78828	52.0		60.7	
		HIGH	HIGH 3-5-2012 5-44-59 PM.SMS.D	Sample		3/5/2012 3:45 PM		9.658	93817		478.5292	478.5292		44.0		31.9		9.933	80580	51.1		63.4	
		BLK	BLK 3-5-2012 9-01-38 PM.SMS.D	Sample		3/5/2012 7:01 PM																	
		208 BLOOD	208 BLOOD 3-5-2012 9-23-28 PM.SMS.D	Sample		3/5/2012 7:23 PM												9.932	69336	55.1		64.9	
		1027 BLOOD	1027 BLOOD 3-5-2012 9-45-10 PM.SMS.D	Sample		3/5/2012 7:45 PM												9.934	67319	52.0		61.0	
		1027 URINE	1027 URINE 3-5-2012 10-07-10 PM.SMS.D	Sample		3/5/2012 8:07 PM												9.933	89998	56.1		58.9	

Note tags for outliers and below calibration.

Naphyrone Calibration



500 ng/mL Standard



Batch Results

		Sample					Naphryro...	Naphryrone Results							Qualifier...		Qualifier...		Ropivacaine(...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		CAL1	CAL1 3-5-2012 3-12-22 PM.SMS.D	Cal	1	3/5/2012 1:12 PM	50.0000	10.364	4481		24.5910	24.5910	49.2	43.8		34.1		9.935	87567	53.7		60.2	
		CAL2	CAL2 3-5-2012 3-34-07 PM.SMS.D	Cal	2	3/5/2012 1:34 PM	100.0000	10.360	10230		62.8105	62.8105	62.8	39.1		36.5		9.934	78265	52.1		57.5	
		CAL3	CAL3 3-5-2012 3-55-54 PM.SMS.D	Cal	3	3/5/2012 1:55 PM	250.0000	10.360	31518		180.1532	180.1532	72.1	43.2		37.5		9.931	84069	50.9		59.8	
		CAL4	CAL4 3-5-2012 4-17-44 PM.SMS.D	Cal	4	3/5/2012 2:17 PM	500.0000	10.361	70525		503.1422	503.1422	100.6	41.3		33.1		9.935	67355	54.6		64.6	
		CAL5	CAL5 3-5-2012 4-39-40 PM.SMS.D	Cal	5	3/5/2012 2:39 PM	1000.0000	10.360	214191		1129.3030	1129.3030	112.9	35.4		26.1		9.932	91140	55.1		60.0	
		NEG	NEG 3-5-2012 5-01-23 PM.SMS.D	Sample		3/5/2012 3:01 PM												9.933	77787	51.2		62.3	
		LOW	LOW 3-5-2012 5-23-10 PM.SMS.D	Sample		3/5/2012 3:23 PM		10.360	15449		94.1742	94.1742		41.4		34.8		9.931	78828	52.0		60.7	
		HIGH	HIGH 3-5-2012 5-44-59 PM.SMS.D	Sample		3/5/2012 3:45 PM		10.362	85630		510.6440	510.6440		41.4		30.5		9.933	80580	51.1		63.4	
		BLK	BLK 3-5-2012 9-01-38 PM.SMS.D	Sample		3/5/2012 7:01 PM																	
		208 BLOOD	208 BLOOD 3-5-2012 9-23-28 PM.SMS.D	Sample		3/5/2012 7:23 PM												9.932	69336	55.1		64.9	
		1027 BLOOD	1027 BLOOD 3-5-2012 9-45-10 PM.SMS.D	Sample		3/5/2012 7:45 PM												9.934	67319	52.0		61.0	
		1027 URINE	1027 URINE 3-5-2012 10-07-10 PM.SMS.D	Sample		3/5/2012 8:07 PM												9.933	89998	56.1		58.9	

Note tags for outliers and below calibration.

Conclusions

This application note presents a sensitive, selective, and robust method to determine pyrovalerone analogs in biological samples using ropivacaine as an internal standard. For the analysis of pyrovalerone analogs, the benefits of GC Quadrupole Ion Trap MS\MS cannot be underestimated. In terms of reducing sample matrix interference, improving signal-to-noise, and coupling its high selectivity and sensitivity, the GC Quadrupole Ion Trap MS\MS provides a more confidence driven solution for the analysis of pyrovalerone analogs. GC Quadrupole Ion Trap MS\MS analysis has the potential to reduce false positive and negatives as well as providing an additional degree of confidence in the results obtained. Using the optimized method listed above, a fast, targeted GC/MS/MS method can be used to solve the current pyrovalerone Analog analysis problem facing forensic laboratories. Positive controls were used in conjunction with negative controls to assure accurate quantification and rule out false negatives in the unknown biological samples. Low ng/mL detection limits were observed for pyrovalerone analogs in various sample matrices.

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Acknowledgement

Saint Louis University Forensic Toxicology Laboratory for providing the data used in this study.

For More Information

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