Differential Recovery of Gabapentin and Pregabalin.

UTILIZING SPE EXTRACTION DEMONSTRATED IN A 43 ANALYTE URINE CONFIRMATORY LC-MS/MS PANEL

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INTRODUCTION

Gabapentin (1-(aminomethyl)cyclohexane acetic acid) and pregabalin (β-isobutyl-γ-Aminobutyric acid) are used primarily to treat seizures and neuropathic pain. Both are recommended as a first line agent for the treatment of pain associated with diabetic neuropathy, postherpetic neuralgia, and central neuropathic pain by the European Federation of Neurological Societies. As demand for therapeutic drug monitoring rises, methods that can be used to quantify a wide variety of drug chemistries from a single analysis are increasingly being implemented as they reduce costs and streamline workflow.

An impediment to the easy implementation of these methods is the inclusion of gabapentin (and to a somewhat lesser extent pregabalin) to these comprehensive methods, as urine specimens routinely have concentrations in the hundreds of microgram per milliliter range, with some samples having concentrations a hundred fold greater. The resulting off-scale chromatographic peaks, complicate automatic detection of these analytes, result in potential carry-over, and can be the cause of ion suppression in chromatographic neighboring analytes.

In addition to the other benefits associated with solid phase extraction; the concentration and types of organic solvents used in the elution step can be modified to predictably and reproducibly reduce the recovery of gabapentin and pregabalin, while not affecting the complete recovery of analytes requiring high levels of sensitivity (buprenorphine, norbuprenorphine, fentanyl, sufentanil, etc.).

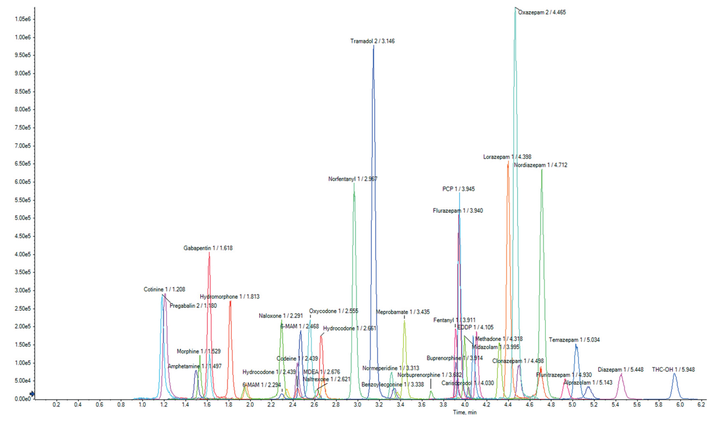

Figure 1: *Example Chromatogram*

ANALYTICAL CONDITIONS

LC Conditions:
Shimadzu Nexera XR UHPLC
Column: RaptorTM Biphenyl, 50mm x 2.1mm, 2.7um, with Raptor
Biphenyl 5mm guard column, Restek Corp., Bellefonte, PA
Flow: 700 µL/min
Column temperature: 40 °C
Injection volume: 5 µL
A=0.1% aqueous formic acid (FA);
B=Methanol w/ 0.1% FA

MS Conditions:
SCIEX QTRAP® 5500, Source=ESI
Positive ion, scheduled MRM
Source temperature 600 °C

LIST OF ANALYTES (43)

6-MAM	Hydrocodone	Norbuprenorphine
Alprazolam	Hydromorphone	Nordiazepam
Amphetamine	Hydroxyalprazolam	Norfentanyl
Benzoylecgonine	Lorazepam	Normeperidine
Buprenorphine	MDA	Oxazepam
Carisoprodol	MDEA	Oxycodone
Clonazepam	MDMA	Oxymorphone
Codeine	Meperidine	PCP
Cotinine	Meprobamate	Pregabalin
Diazepam	Methadone	Sufentanil
EDDP	Methamphetamine	Temazepam
Fentanyl	Midazolam	THC-OH
Flunitrazepam	Morphine	Tramadol
Flurazepam	Naloxone	
Gabapentin	Naltrexone	

RESULTS

- All analytes with the exception of Benzoylecgonine, Gapapentin & Pregabalin had recoveries greater than 90% for all elution buffers tested.
- Benzoylecgonine had poor recovery for the elution solvent combination of ethyl acetate & 2-propanol (less than 50%); the combination of dichloromethane & 2-propanol showed good recovery.
- Gabapentin & Pregabalin had a maximum recovery of approximately 9% with elution solvent combination of ethyl acetate & 2-propanol; the extracts were clear and colorless.
- Gabapentin & Pregabalin had reproducible recoveries ranging from 8 %to 37% with the elution solvent combination of dichloromethane & 2-propanol; the extracts were clear and colorless.

EXPERIMENTS

A absolute recovery experiment was performed with a variety of elution buffers with combinations of ethyl acetate (EtOAc), dichlormethane (DCM), 2-propanol (IPA), and methanol (MeOH).

The procedure in brief is as follows:

150 µL of Master Mix (containing 120 µL of 100mM sodium acetate pH 4.8, 20 µL of internal standard mix, and 10 µL of β-glucuronidase solution (Kura BG100 at 100,000 units/mL) were added to Tecan SP Narrow Bore HP SCX 5mg 96-well plates, followed by 50 µL of blanks, standards, controls and specimens. The hydrolysis reaction was allowed to proceed for 1 hour at 68 °C. The samples were loaded onto the sorbent at flow rates of 1-2 mL/min, the columns were then subsequently washed with 500 µL of deionized water, 200 µL of 100mM hydrochloric acid, and 300 µL of deionized water. The columns were then dried under nitrogen for 5 minutes and eluted into a collection plate containing internal standard. Samples were then dried under nitrogen and resuspended with 200 µL of 95:5 Mobile Phase A:B.

SUMMARY & CONCLUSIONS

Using various combinations of ethyl acetate, 2-propanol, dichloromethane & methanol; the recovery of gabapentin & pregabalin can be predictably and reproducibly adjusted from 8% to complete recovery while not impacting the complete recovery of most other analytes.

This differential recovery can reduce the complications associated with samples with high concentration gabapentin/pregablin such as offscale chromatography, carry over & ion suppression.

BENZOYLECGONINE RECOVERY

Elution Solvents	% Recovery Rep 1	Rep 2	Rep 3	Mean	SD	% C.V.
90:08:02 EtOAc:IPA:NH4OH	3.7	3.6	4.4	**3.9**	0.42	10.6
80:18:02 EtOAc:IPA:NH4OH	10.4	15.6	50.7	**25.6**	21.94	85.8
70:28:02 EtOAc:IPA:NH4OH	25.1	32.8	24.8	**27.6**	4.54	16.5
60:38:02 EtOAc:IPA:NH4OH	37.5	55.8	52.5	**48.6**	9.77	20.1
50:48:02 EtOAc:IPA:NH4OH	42.8	47.9	44.7	**45.2**	2.59	5.7
90:08:02 EtOAc:MeOH:NH4OH	37.9	31.7	41.3	**36.9**	4.89	13.2
80:18:02 EtOAc:MeOH:NH4OH	88.7	91.8	97.3	**92.6**	4.38	4.7
70:28:02 EtOAc:MeOH:NH4OH	88.8	100.1	92.6	**93.8**	5.73	6.1
60:38:02 EtOAc:MeOH:NH4OH	93.3	93.8	98.7	**95.3**	2.98	3.1
50:48:02 EtOAc:MeOH:NH4OH	94.5	98.7	94.4	**95.9**	2.49	2.6
90:08:02 DCM:IPA:NH4OH	97.6	96.8	86.6	**93.7**	6.11	6.5
80:18:02 DCM:IPA:NH4OH	102.4	102.7	101.6	**102.2**	0.56	0.5
70:28:02 DCM:IPA:NH4OH	98.5	91.8	98.8	**96.4**	3.95	4.1
60:38:02 DCM:IPA:NH4OH	97.5	91.6	92.1	**93.7**	3.22	3.4
50:48:02 DCM:IPA:NH4OH	92.2	90.7	87.1	**90.0**	2.60	2.9
90:08:02 DCM:MeOH:NH4OH	88.5	97.8	95.5	**93.9**	4.86	5.2
80:18:02 DCM:MeOH:NH4OH	95.0	95.4	94.8	**95.1**	0.34	0.4
70:28:02 DCM:MeOH:NH4OH	90.8	97.0	91.7	**93.2**	3.36	3.6
60:38:02 DCM:MeOH:NH4OH	92.3	101.0	94.2	**95.8**	4.55	4.7
50:48:02 DCM:MeOH:NH4OH	98.5	95.6	91.9	**95.3**	3.31	3.5

GABAPENTIN RECOVERY

Elution Solvents	% Recovery Rep 1	Rep 2	Rep 3	Mean	SD	% C.V.
90:08:02 EtOAc:IPA:NH4OH	0.2	0.3	0.6	**0.4**	0.19	52.7
80:18:02 EtOAc:IPA:NH4OH	0.1	0.2	1.4	**0.6**	0.70	123.0
70:28:02 EtOAc:IPA:NH4OH	1.2	1.3	1.1	**1.2**	0.14	11.3
60:38:02 EtOAc:IPA:NH4OH	2.8	9.9	7.5	**6.7**	3.64	54.1
50:48:02 EtOAc:IPA:NH4OH	7.3	9.4	9.0	**8.6**	1.14	13.3
90:08:02 EtOAc:MeOH:NH4OH	0.6	0.4	0.7	**0.6**	0.19	33.2
80:18:02 EtOAc:MeOH:NH4OH	17.6	18.5	24.8	**20.3**	3.89	19.2
70:28:02 EtOAc:MeOH:NH4OH	77.8	76.7	80.7	**78.4**	2.09	2.7
60:38:02 EtOAc:MeOH:NH4OH	92.9	96.4	96.1	**95.1**	1.92	2.0
50:48:02 EtOAc:MeOH:NH4OH	94.4	96.0	95.1	**95.2**	0.82	0.9
90:08:02 DCM:IPA:NH4OH	0.4	0.2	0.5	**0.4**	0.16	41.2
80:18:02 DCM:IPA:NH4OH	7.3	7.6	6.7	**7.2**	0.44	6.2
70:28:02 DCM:IPA:NH4OH	18.4	20.4	17.3	**18.7**	1.56	8.3
60:38:02 DCM:IPA:NH4OH	23.9	23.4	27.7	**25.0**	2.35	9.4
50:48:02 DCM:IPA:NH4OH	37.9	35.9	38.1	**37.3**	1.24	3.3
90:08:02 DCM:MeOH:NH4OH	31.3	30.8	25.6	**29.2**	3.17	10.8
80:18:02 DCM:MeOH:NH4OH	97.8	95.3	98.6	**97.2**	1.71	1.8
70:28:02 DCM:MeOH:NH4OH	95.5	97.2	93.8	**95.5**	1.68	1.8
60:38:02 DCM:MeOH:NH4OH	97.0	97.7	98.1	**97.6**	0.57	0.6
50:48:02 DCM:MeOH:NH4OH	98.7	97.5	96.2	**97.4**	1.28	1.3

PREGABALIN RECOVERY

Elution Solvents	% Recovery Rep 1	Rep 2	Rep 3	Mean	SD	% C.V.
90:08:02 EtOAc:IPA:NH4OH	2.5	2.3	2.3	**2.4**	0.08	3.3
80:18:02 EtOAc:IPA:NH4OH	2.0	1.8	2.1	**2.0**	0.11	5.7
70:28:02 EtOAc:IPA:NH4OH	1.3	1.9	1.1	**1.4**	0.44	31.2
60:38:02 EtOAc:IPA:NH4OH	1.7	12.8	9.9	**8.1**	5.73	70.5
50:48:02 EtOAc:IPA:NH4OH	8.0	9.6	9.6	**9.0**	0.95	10.5
90:08:02 EtOAc:MeOH:NH4OH	1.5	1.4	1.4	**1.4**	0.02	1.1
80:18:02 EtOAc:MeOH:NH4OH	19.6	19.9	28.9	**22.8**	5.29	23.2
70:28:02 EtOAc:MeOH:NH4OH	80.2	78.0	81.9	**80.0**	1.97	2.5
60:38:02 EtOAc:MeOH:NH4OH	87.3	90.7	91.3	**89.7**	2.15	2.4
50:48:02 EtOAc:MeOH:NH4OH	92.0	90.4	96.3	**92.9**	3.02	3.3
90:08:02 DCM:IPA:NH4OH	1.6	0.8	0.7	**1.1**	0.48	45.6
80:18:02 DCM:IPA:NH4OH	9.1	6.8	7.2	**7.7**	1.22	15.9
70:28:02 DCM:IPA:NH4OH	18.4	21.5	17.7	**19.2**	2.00	10.4
60:38:02 DCM:IPA:NH4OH	23.0	24.8	30.3	**26.0**	3.79	14.6
50:48:02 DCM:IPA:NH4OH	38.5	36.0	35.3	**36.6**	1.65	4.5
90:08:02 DCM:MeOH:NH4OH	25.7	27.2	20.8	**24.5**	3.35	13.7
80:18:02 DCM:MeOH:NH4OH	93.4	91.5	93.7	**92.9**	1.19	1.3
70:28:02 DCM:MeOH:NH4OH	92.7	94.7	93.2	**93.6**	1.03	1.1
60:38:02 DCM:MeOH:NH4OH	90.5	92.4	95.5	**92.8**	2.50	2.7
50:48:02 DCM:MeOH:NH4OH	95.3	93.1	94.3	**94.2**	1.10	1.2