Validation of Screening Methods and Reportable Qualitative Methods

Purpose	To demonstrate that the method is valid for casework on a secondary GC/MS system.
Analyte	GCMS Drug Screen and Qualitative Confirmation
Units of Measure	ng/mL
Analyst Performing Validation Studies	F. Bayless, D. Mike, M. Rodriguez
Responsible Supervisor	Melissa Henry
Start Date	April 19, 2022
Completion Date	June 9, 2022
Primary Matrix	Urine
Secondary Matrix	N/A
Cut-off Calibrator	Norketamine (50 ng/mL), Diphenhydramine (25 ng/mL), Doxylamine (25 ng/mL), Tramadol (10 ng/mL), Chlorpheniramine (10 ng/mL), Venlafaxine (50 ng/mL), Brompheniramine (10 ng/mL), Methadone (50 ng/mL), Dextromethorphan (25 ng/mL), Amitriptyline (25 ng/mL), Imipramine (10 ng/mL), Cyclobenzaprine (10 ng/mL), Sertraline (25 ng/mL), and Zolpidem (10 ng/mL)
Equipment/Instrument	GCMS-6
Instrument Serial Number	US2202M007
Method	BSD.M

Validation Approval

Analyst: Fredria Bayless Digitally signed by Fredria Bayless Date: 2022.08.25 13:02:36 -05'00' 08/25/2022
Date

Analyst: Melissa Rodriguez Digitally signed by Melissa Rodriguez DN: cn=Melissa Rodriguez, o=Houston Forensic Science Center, ou=Toxicology, email=mrodriguez@houstonforensicscience.org, c=US Date: 2022.08.25 13:22:07 -05'00' 08/25/2022
Date

Analyst: Dana R. Mike Digitally signed by Dana R. Mike Date: 2022.08.26 10:46:11 -05'00' 08/26/2022
Date

Responsible Supervisor: Melissa Henry Melissa Henry cn=Melissa Henry, o=Houston Forensic Science Center, ou=Toxicology, email=mhenry@hfsctx.gov, c=US 2022.08.29 08:57:40 -05'00' 08/29/2022
Date

Quality Division: Jackeline Moral Digitally signed by Jackeline Moral Date: 2022.08.31 13:20:58 -05'00' 08/31/2022
Date

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: GCMS Drug Screen and Qualitative Confirmation
Units: ng/mL
Method: BSD.M
Instrument: GC/MS-6
SOP Reference: Toxicology Analytical Manual v3.8

Analyst: FB, DM, MR
Study Dates: 04/19/2022 to 06/09/2022
Matrix: Urine

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Cut-off Match %	All cut-off samples must have a match greater than or equal to 60%	All cut-off samples showed acceptable match at the cut-off concentration.	Sertraline did not pass in BSD_20220419U_MR for Cut-off 3.
1	Positive Control Match %	All positive conrtol samples must have a match greater than or equal to 60%	All positive control samples showed acceptable match at double the cut-off concentration.	Sertraline was not above the cut-off concentration for POS 2 in BSD_20220419U_MR.

Validation Study

Cut-off Match Percentage

Analyte: GCMS Drug Screen and Qualitative Confirmation
Units: ng/mL
Instrument: GC/MS-6

Analyst: FB, DM, MR
Study Dates: 04/19/2022 to 06/09/2022
Matrix: Urine

Batch Name	Drug	Cut-off 1		Cut off 2		Cut off 3	
		% Match	LOD response	% Match	LOD response	% Match	LOD response
BSD_20220419U_MR	Norketamine	95	16660	95	19600	95	12498
	Diphenhydramine	86	8798	88	10893	87	6353
	Doxylamine	85	4799	84	6645	81	3221
	Tramadol	77	2177	83	3714	69	1527
	Chlorpheniramine	92	4338	92	5725	92	2605
	Venlafaxine	80	14091	81	23474	80	9571
	Brompheniramine	91	2345	91	3151	89	1474
	Methadone	73	35599	73	40334	72	22085
	Dextromethorphan	87	4581	87	5485	87	2826
	Amitriptyline	82	13738	83	16906	82	8884
	Imipramine	92	1604	93	2363	90	1176
	Cyclobenzaprine	85	4708	84	6046	82	2799
	Sertraline	83	4311	82	4989	81*	1683*
	Zolpidem	90	4040	92	4291	88	3396

Batch Name	Drug	Cut-off 1		Cut off 2		Cut off 3		Cut off 4	
		% Match	LOD response	% Match	LOD response	% Match	LOD response	% Match	LOD response
BSD_20220609U_FB	Norketamine	79	10569	77	9676	73	11078	95	10603
	Diphenhydramine	87	6393	86	6036	85	6031	89	6833
	Doxylamine	85	4127	84	3828	84	3849	86	4210
	Tramadol	82	2801	82	2596	83	2596	82	2867
	Chlorpheniramine	92	3408	91	2964	92	3068	89	3250
	Venlafaxine	83	17228	83	16125	83	15934	83	17685
	Brompheniramine	91	1833	91	1713	92	1761	92	1933
	Methadone	73	22582	73	20818	73	20894	73	22974
	Dextromethorphan	87	2890	87	2522	86	2584	85	2598
	Amitriptyline	86	10645	86	9825	85	9929	85	10469
	Imipramine	93	1534	92	1341	93	1387	93	1515
	Cyclobenzaprine	84	3921	86	3602	84	3602	85	3853
	Sertraline	85	2407	85	2246	85	2281	85	2802
	Zolpidem	91	4615	90	4157	92	4312	91	4443

Batch Name	Drug	Cut-off 5	
		% Match	LOD response
BSD_20220609U_FB	Norketamine	95	11261
	Diphenhydramine	88	6400
	Doxylamine	84	4137
	Tramadol	83	2624
	Chlorpheniramine	89	3278
	Venlafaxine	82	17481
	Brompheniramine	93	1888
	Methadone	74	22721
	Dextromethorphan	85	2718
	Amitriptyline	85	10557
	Imipramine	93	1491
	Cyclobenzaprine	86	3989
	Sertraline	85	2850
	Zolpidem	91	4704

Batch Name	Drug	Cut-off 1		Cut off 2	
		% Match	LOD response	% Match	LOD response
BSD_20220516U_DM	Norketamine	95	8239	96	16904
	Diphenhydramine	88	3968	89	8983
	Doxylamine	85	2308	85	5593
	Tramadol	81	1159	83	3420
	Chlorpheniramine	86	2053	92	5031
	Venlafaxine	79	7436	80	21717
	Brompheniramine	91	1171	92	3089
	Methadone	71	13603	72	31651
	Dextromethorphan	84	2113	86	4989
	Amitriptyline	80	5501	82	13632
	Imipramine	92	870	93	2219
	Cyclobenzaprine	82	1930	84	4696
	Sertraline	84	1581	85	5172
	Zolpidem	91	2021	93	7239

Results: All cut-off samples showed acceptable match at the cut-off concentration.
Comments: Sertraline did not pass in BSD_20220419U_MR for Cut-off 3.

Acceptance Criteria: **All Cut-off Calibrator Samples must have a percent match greater than or equal to 60%.**

Validation Study

Positive Match Percentage

Analyte: GCMS Drug Screen and Qualitative Confirmation
Units: ng/mL
Instrument: GC/MS-6

Analyst: FB, DM, MR
Study Dates: 04/19/2022 to 06/09/2022
Matrix: Urine

Drug	BSD_20220419U_MR			BSD_20220516U_DM			BSD_20220609U_FB			
	POS 1	POS 2	POS 3	POS 1	POS 2	POS 3	POS 1	POS 2	POS 3	POS 4
Norketamine	96	96	96	96	96	97	86	97	97	96
Diphenhydramine	86	88	89	87	88	89	88	89	90	90
Doxylamine	84	83	84	84	85	85	84	84	85	85
Tramadol	78	83	84	81	82	82	85	85	84	85
Chlorpheniramine	91	93	93	91	92	91	94	93	92	94
Venlafaxine	82	82	82	80	80	81	83	84	83	83
Brompheniramine	92	92	92	91	92	92	92	92	93	93
Methadone	74	73	73	72	73	72	74	73	74	74
Dextromethorphan	86	87	87	85	86	86	87	87	87	86
Amitriptyline	85	85	86	83	84	84	87	87	88	88
Imipramine	92	92	93	93	92	93	93	93	93	93
Cyclobenzaprine	81	84	85	82	83	84	86	85	86	85
Sertraline	86	82*	83	85	85	85	86	86	86	86
Zolpidem	93	91	92	91	92	93	92	92	92	92

Results: All drugs in positive controls are present and above the cut-off concentrations
Comments: Sertraline was not above the cut-off concentration for POS 2 in BSD_20220419U_MR.

Acceptance Criteria: **All positive control samples must have a match greter than or equal to 60%**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: GCMS Drug Screen and Qualitative Confirmation
Units: ng/mL
Instrument: GC/MS-6
Analyst: FB, DM, MR
Study Dates: 04/19/2022 to 06/09/2022
Matrix: Urine

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

Date	Reason
April 26, 2022	Mepivacaine internal standard response is overloaded for all known samples, therefore, this batch fails to meet acceptance criteria.

Deviations from SOP: N/A

Other Observations: Not all validation parameters were assessed in this urine validation because it was considered to be an extension of the full validation in urine previously performed. The evaluation of the secondary instrument was abbreviated to cut-off match percentage and positive control match percentage.

Sample Preparation Steps: Refer to Toxicology Analytical Manual v3.8, "Drug Screen and Qualitative Confirmation by Gas Chromatography-Mass Spectrometry" section titled "Extraction Procedure".

Location of Raw Data: Toxicology section shared electronic storage.

Recommended Maximum Run Length (# unknown samples): 20

Conclusion: This method is fit for use on casework for GCMS drug screen and qualitative confirmation analyses in urine.