How to improve the precision of analysis - semi -automated sample preparation for psychoactive compounds quantification in human plasma

Maciej Stopa[1,2], Julia Mironenka[1], Anna Lenartowicz[1], Adrian Soboń[1,2], Rafał Szewczyk[1,2], Katarzyna Krupczyńska-Stopa[1,2], Andrzej Kwaśnica[3]

1. LabExperts sp. z o. o., Gdańsk, Poland; 2. Bioanalytic sp. z o. o., Gdańsk, Poland; 3. Lab4Tox sp. z o. o., Wrocław, Poland
The authors declare no competing financial interest.

Complex and rigorous sample preparation is required to obtain accurate detection of psychoactive compounds in the multimethod and to quantitatively determine their presence.

Chromatography run was performed using ExionAC equipped with Fortis H2O C18 column. The method was run in reverse phase mode, using as mobile phases acidified with formic acid both, water and acetonitrile. Mass spectrometry was performed using ZenoTOF 7600 (SCIEX), with following conditions: positive electrospray scheduled MRMhr mode acquiring full MS/MS spectra.

Sample preparation procedure using Hudson SOLO automatic pipette station include deproteinization and extraction, dissolution in 100 minutes.

Figure 1: Semi-automated sample preparation protocol using Hudson SOLO automatic pipette station with Hudson SOLO Soft and SoftLink V Protocol Editor.
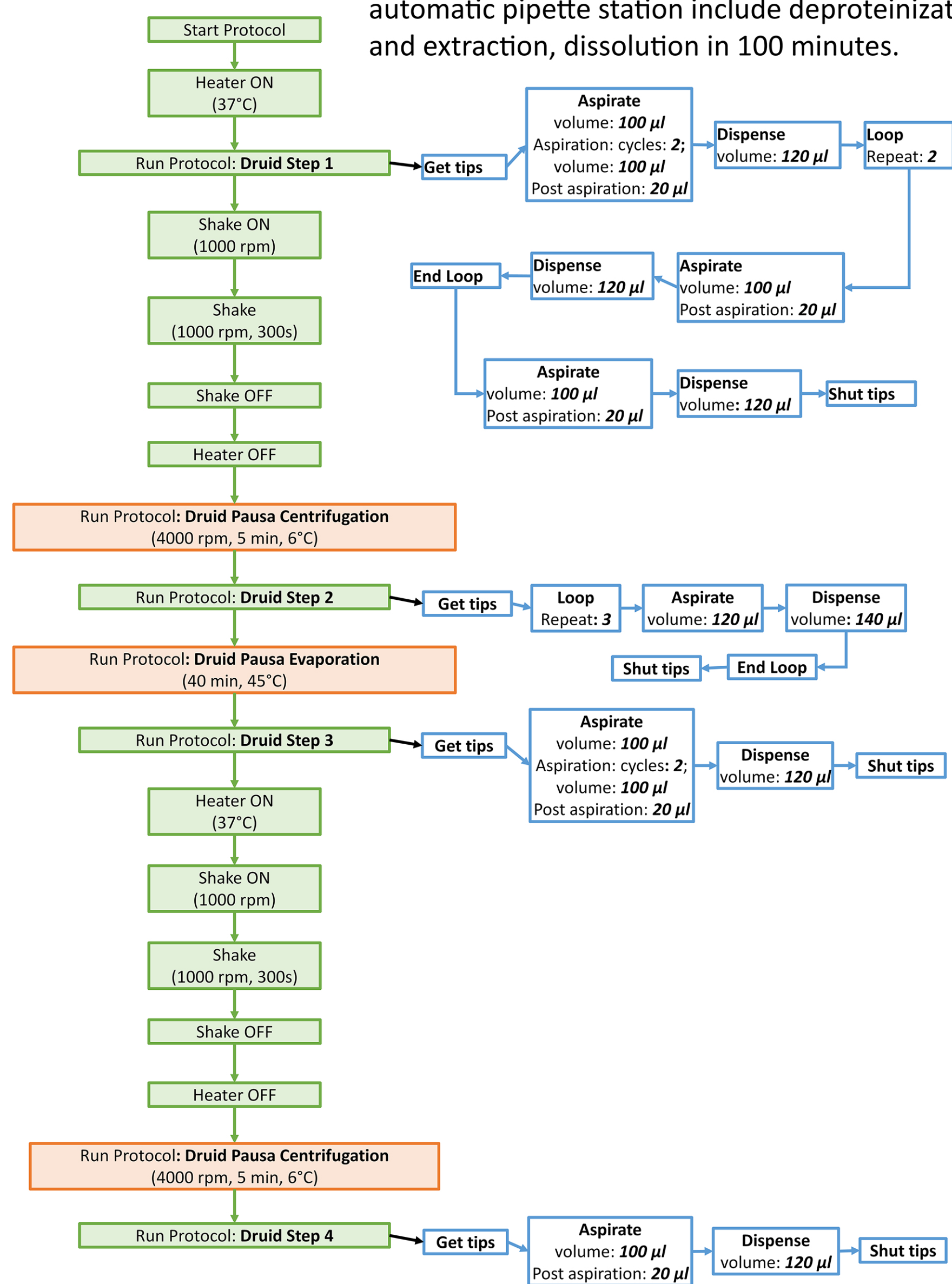
Table 1. Part A: The comparison of manual vs-semi automated sample preparation precision; Part B: The results of method validation of semi-automated sample preparation.

A [2.5 ng/ml] (n=6)			B Validation results		
Manual method precision CV [%]	Semi-automated method precision CV [%]	Analyte	Linearity range [ng/ml]	R	LOD [ng/ml]
14.51	7.76	6-monoacetylomorphine	0.1-50	0.99656	0.1
14.81	9.27	7-aminoflunitrazepam	0.1-50	0.99729	0.01
12.38	8.33	7-aminoclonazepam	0.1-50	0.99556	0.1
8.62	5.26	Alprazolam	0.1-50	0.99888	0.025
9.49	6.44	Amphetamine	0.25-50	0.99868	0.1
N/A	11.32	Amitriptyline	0.1-50	0.99836	0.025
13.19	7.21	Benzoylecgonine	0.1-50	0.99821	0.0025
N/A	11.20	Desipramine	0.1-50	0.99883	0.01
8.47	7.05	Diazepam	0.1-50	0.99805	0.025
N/A	8.22	Doxepin	0.1-50	0.99710	0.025
9.52	7.53	Fentanyl	0.1-50	0.99829	0.0025
10.92	9.44	Flunitrazepam	0.1-50	0.99896	0.025
11.41	8.31	Hydroxyzine	0.1-50	0.99735	0.01
N/A	13.55	Imipramine	0.1-50	0.99593	0.01
N/A	8.59	Klomipramina	0.1-50	0.99875	0.025
13.24	9.27	Clonazepam	0.1-50	0.99801	0.1
8.67	8.90	Codeine	0.1-50	0.99641	0.1
9.70	6.39	Cocaine	0.1-50	0.99903	0.0025
11.30	10.96	Lorazepam	0.1-50	0.99816	0.025
13.15	9.50	MDA	0.1-50	0.99764	0.025
12.16	12.28	MDEA	0.1-50	0.99844	0.0025
10.13	11.58	MDMA	0.1-50	0.99898	0.01
4.80	6.66	Methadone	0.1-50	0.99742	0.0025
13.54	12.78	Methamphetamine	0.1-50	0.99502	0.01
10.00	7.00	Morphine	0.1-50	0.99904	0.1
9.65	8.82	Nordiazepam	0.1-50	0.99945	0.025
N/A	8.00	Nortriptyline	0.1-50	0.99593	0.025
10.44	8.43	Oxazepam	0.1-50	0.99819	0.025
N/A	7.03	Opipramol	0.1-50	0.99883	0.025
6.05	6.81	THC	0.1-50	0.99747	0.1
14.69	14.96	THC-COOH	0.25-50	0.99733	0.25
12.49	11.76	Tramadol	0.1-50	0.99796	0.001
5.10	4.71	Zolpidem	0.1-50	0.99863	0.0025
12.61	7.11	Zopiclone	0.1-50	0.99793	0.01

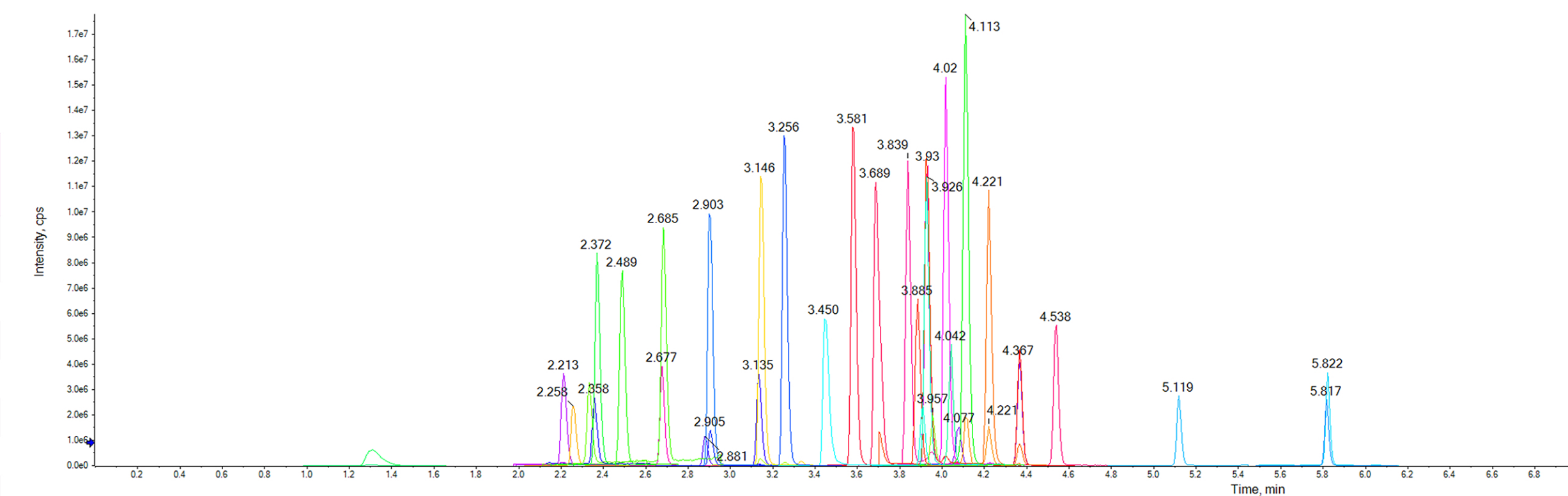N/A – analytes have not been included in previous version of analytical method

Figure 2: Chromatographic separation of analytes included in multi-method of psychoactive compunds quantitative determination [c=25 ng/ml].

Table 2. Ion source parameters.

Ion source parameters		POS
	CUR	35
	CAD	7
	IS	5000
	TEM	600
	GS1	60
	GS2	60

Table 3. LC method device parameters.

		Stop time:	10.00 min	
		Flow:	0.5000 mL/min	
LC gradient		Time (min)	Flow (ml/min)	B Conc (%)
		0	0.500	0
		0.5	0.500	0
		6	0.500	100
		8	0.500	100
		8.1	1.000	0
		9	1.000	0
		10	0.500	0
		Compressibility settings (GPa):		
Mobile phase A	Water		0.45	
Mobile phase B	Acetonitrile		1.2	
		Injection		10 µL
		Sampling speed:		5 µL/s
		Use cooler temperature:		Yes
		Coler temperature:		8 °C
		Column Oven		45 °C

Conclusion

Designed LC-MS/MS analysis include determination of 35 psychoactive compounds in one method, lasting 10 minutes from complex matrix (human plasma).

Previously desinged manual method was moved to a higher level, in order to give cinsistent result using semi-atomated sample preparation

Sample preparation procedure using Hudson SOLO automatic pipette station works precisely, flawlessly and