Electronic Supplementary Material (ESI) for Analytical Methods.

Versatile additively manufactured (3D printed) wall-jet flow cell for high performance liquid chromatography- amperometric analysis: Application to the detection and quantification of New Psychoactive Substances (NBOMes)

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Table S1. Scientific articles found in literature that have utilized different types of flow cells for HPLC-AD analysis and the advantages and disadvantages of each platform.

Flow cell type/design	Flow cell material	Working electrode	Analyte detected	Advantage of the design	Disadvantage of the design	Ref.
Three pin tubular flow cell	Epoxy resin and teflon	Pt, C and Au	p-aminophenol and p-nitrophenol	- Increased sensitivity. - Lower effect of flow-rate on current measurement -Increase in electrode stability for the short-term sampling period and for extended routine operation. - Decreased noise levels.	- Faster scan rates distort and shift the waves, making them unsuitable for quantitative analysis but only adequate for qualitative purposes. - The cell is fragile, subject to leaks and had non-reproducible electrode characteristics from cell to cell. -Limited applicable solvent systems.	1
Wall-jet flow cell based on rotating disk electrode(RDE)	Δ[a]	Home-made carbon paste	Potassium hexacyanoferrate(II)	- Increased sensitivity.	- The peak shows some tailing.	2
Wall-jet flow cell based on rotating disk electrode	Δ	Home-made carbon paste (35% Dow Corning high vacuum silicone grease and 65% graphite powder)	Catecholamines (Dopamine, noradrenaline and 3,4-dihydroxyphenylacetic acid)	- Increased detector response. - No peak broadening.	- Deviations with the ideal behavior of the RDE due to wall effects in the limited volume of the flow cell.	3
Wall-jet flow cell	Δ	GC[b] disc, DME[c]	Chlormadinone, estrone, p-nitrophenol, amino acids and citral	- High sensitivity, the cell is capable of detecting a wide range of compounds down to picogram levels. - Small cell volume that could be varied and easily maintained. - Wide operating voltage range. - Non influence of surface absorption. - The pulse polarographic measuring mode can operate over a large linear concentration	Δ	4

				range.		
Rectangular channel-type cell	Polytetrafluoroethylene and stainless steel	Carbon paste (graphite powder and polypropylene)	Hydroquinone	Δ	Δ	[5]
Simple design based on wall-jet principle	Teflon	GC, carbon paste (graphite powder and nujol) and Hg	Benzodiazepines (nitrazepam, diazepam and chlordiazepoxide)	- Interchangeable working electrodes. - Satisfactory response even when the mobile phase has low electrical conductivity. - Minimized resistance. - Cheaper and can be more selective than other sensitive detectors (e.g. UV[d]).	-When the detector is used in the reduction mode, interferences might occur from the reduction of oxygen, hydrogen ions and trace metals. -Constant-potential amperometry was found to be preferable to normal potential pulse technique, which gave high background currents when solid electrodes were used. - Differential pulse only detects compounds with half-wave potentials close to the initial potential chosen.	[6]
Optically transparent thin-layer electrochemical flow cell	Teflon	Au minigrid	A radiopharmaceutical analogue consisting of a technetium-99-hydroxyethylidene diphosphonate (HEDP) complex mixture	- Minimum interference. - Decreased the amount of sample handling, which is crucial for toxic or radioactive samples. -The cell can be used for thin-layer cyclic voltammetry, coulometry, and spectropotentiostatic characterization in the stop-flow mode and it could be used as a spectrophotometric or an electrochemical detector in a dynamic mode. -The cell can be used for HPLC by the employment of a low volume bypass switching valve.	Δ	[7]
					- The device works best for chemically reversible analytes rather than irreversible ones.	

					- When an electrode is in the "off" state, its potential drops, and when it returns to the "on" state, the auxiliary electrode is required to drive a large current through the solution resistance to charge the double-layer capacitance. This may take a relatively long time. - Because of the long time constant for operation in the alternate switching mode, the sensitivity is low and the peaks are broad.	
Large volume wall-jet cells	1st flow cell (disc electrode): Kel-F. 2nd flow cell (ring electrode): stainless steel. 3rd flow cell: Teflon and glass.	GC	- Hydralaxine and its metabolites. - Phenols. - Hydroquinone.	- Easy to fabricate the cell. - Similar sensitivity to UV detector. - Negligible loss of resolution. - Large volume flow cell: suitable for aqueous and non-aqueous work (effective for gradient-elution), and the electrode system is visible.	- The peak heights change substantially as the inlet electrode distance is increased.	9
Large volume wall-jet cell	Plexiglass and stainless-steel	Silicone rubber-impregnated carbon electrode.	Epinephrine, dopamine, vanillylmandelic acid, dopamine, 5-hydroxytryptophan, norepinephrine, 5-hydroxyindoleacetic acid, homovanillic acid, dihydroxynorepinephrine, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylglycol and dihydroxybenzylamine	- Easy to build and to handle. - Can accommodate any commercial working, reference and auxiliary electrodes. - Silicone rubber-impregnated carbon electrode is more sensitive than GC and silicon-based carbon paste electrodes, more robust, inexpensive and can be made in the lab at any size and shape.	Δ	10
Microdisk electrochemical flow-jet (wall-jet)	Δ	Carbon and Pt microelectrodes	Ferrocene	- Enhanced signal-to-noise ratios. - Microsensors function very well in the highly resistive media associated with dilute electrolyte and organic solvents. - Possibility of elimination of potentiostat in	Δ	11

				case of carbon microelectrode. - Minimized flow-rate dependence. - Possible surface electrode cleaning.		
Laminar flow pattern flow cell	Polyacrylic acid	Rotating disc electrode made from solid paraffin (40%; wt/wt) and graphite powder (60%; wt/wt)	Biogenic monoamines and their metabolites(dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine, noradrenaline, 3-methoxy-4-hydroxyphenyl-ethyleneglycol, 5-HT, and 5-hydroxyindoleacetic acid)	- Excellent sensitivity, good stability of the electrode and easy to use detector.	Δ	12
Micro-electrochemical flow cell	Δ	Carbon fiber-based electrode	Salbutamol	- Simple to prepare and easy to manage. - Less air bubbles and leaks. - Reproducible with better detection limits than GC.	- High noise level.	13
Versatile wall-jet, wall tube or radial-flow thin-layer large volume cell	PTFE[e], stainless-steel and glass	Pt, Au, GC discs, Au-Au ring disc and Pt microelectrode	A mixture of regio-isomers of nitrobenzene derivatives.	- The flow cell can operate either in wall-jet, thin-layer or wall tube arrangement. - Post column derivatization of the analyte or modification of the mobile phase is feasible in the wall-jet arrangement, thus enhancing the selectivity and/or the detection limits. - Thin-layer arrangement can be used to test different working electrode materials as the working electrodes can be readily exchanged.	Δ	14
Wall-jet and low-dead-volume electrochemical cell	Δ	Disposable copper-plated screen-printed carbon electrodes	Urine metabolites (creatinine, cystine, uric acid, oxalic acid, and citric acid)	- Low detection limits. -Since disposable electrodes are used, therefore, polishing and reconditioning are avoided. -Reproducible. - High selectivity of the utilised electrodes.	Δ	15
					-Thin-layer cell was dismantled several times for cleaning, however the wall-jet	

	Teflon, stainless steel.			linear dynamic range and lower quantitation limits. - Wall-jet cell is easier to maintain than the thin-layer cell. -Stable background current, repeatable and stable response of the detected analytes. - Low noise levels.	cell did not require special electrode cleaning. - Wall-jet cell has lower peaks due to lower active area of BDD electrode in this arrangement. -Thin-layer cell has higher noise levels. - The peak symmetry is slightly lower in ED[g] generally than spectrophotometric detectors. -Both arrangements have higher flow rate dependence, especially for the wall-jet arrangement.	
Wall-jet/thin-layer flow cell	Δ	GC	Dopamine and homovanillic acid	- Enhances the analytes responses, which improve the sensitivity. - Simple and facile operation allows for more convenient washing or modification of the working electrode surface. - LOD[h] obtained lower than those with UV and commercial ED.	Δ	17
Wall-jet/thin-layer flow cell	Δ	GC	Isoniazid and rifampicin	- The cell can work in the gradient elution mode and it is noise insensitive. - Great signal-to-noise ratio than the commercial electrochemical cells. - The working electrode can be cleaned easily and is more convenient than in case of the commercial cells. - LOD obtained lower than those with UV and commercial ED. - Combines the advantages of both the thin-layer and wall-jet modes.	Δ	18
Three electrode flow cell	Δ	BDD microelectrodes arrays	Hydroquinone	- BDD worked efficiently in high flow rate conditions. -Easy in situ cleaning of the working electrode. - Sensitivity at the picogram scale.	Δ	19

				- High signal-to-noise ratio.		
3D printed wall-jet flow cell (microchip-based electrochemical detection)	Full Cure 720 material (approximately containing 10–30% isobornyl acrylate, 10–30% acrylic monomer, 15–30% acrylate oligomer, 0.1–1% photo initiator)	GC and platinum	Catechol, dopamine and epinephrine	- Improves mass transport on the electrode surface and its sensitivity is higher than TLE[i]. - Inexpensive electrochemical detector for HPLC. - Reusable and versatile device. - Rapid replacement of electrodes without fabricating a new device. - Inexpensive electrochemical detector. - Similar separation performance to LC-UV[j] and LC-MS[k].	Δ	20
Versatile wall-jet AM/3D printed flow cell for HPLC-AD	Photopolymer resin (Clear FLGPCL02)	SPE, graphite sheet, AM/3D printed electrodes: Proto-Pasta, Black Magic and NG/PLA AM-electrode	NBOMes derivatives	-Simple design. -Inexpensive. -Short manufacturing time - Reusable and versatile and can use a wide range of electrodes. - Rapidly assembled and easy replacement of the electrodes without fabricating a new cell. -Withstands high flow rates without leaks. - Graphite sheets, NA/PLA AM-electrode, SPEs can be easily integrated into the cell, and alongside with the AM/3D printed flow cell they can provide a reliable sensitive analytical sensor for routine quantitative analysis in any quality control lab.	-----	This work

[a]**Δ:** not mentioned; [b]**GC:** glassy carbon electrode; [c]**DME:** dropping mercury electrode; [d]**UV:** ultraviolet detection; [e]**PTFE:** polytetrafluoroethylene; [f]**BDD:** boron doped diamond; [g]**ED:** electrochemical detection; [h]**LOD:** limit of detection; [i]**TLE:** thin-layer electrode; [j]**LC-UV:** liquid chromatography-ultraviolet detection; [k]**LC-MS:** liquid chromatography-mass spectrometric detection.

Table S2. Intraday precision study of NBOMe derivatives performed by the proposed AM/3D printed LC-AD wall-jet flow cell system using the electrodes investigated in this study.

Analyte / Working Electrode		25F-NBOMe	25C-NBOMe	25B-NBOMe	25I-NBOMe
	Concentrations	**(RSD%, *n*=3)**			
SPE (AM/3D printed wall-jet flow cell) Linear range: 20-300 µg/mL	**20 µg/mL**	1.35	2.39	3.01	2.80
	100 µg/mL	3.77	1.18	1.24	3.11
	200 µg/mL	1.67	2.77	0.70	2.57
SPE (commercial impinging jet flow cell) [21] Linear range: 20-300 µg/mL	**20 µg/mL**	0.74	0.45	1.84	0.83
	100 µg/mL	0.79	0.77	0.69	1.12
	200 µg/mL	0.72	0.24	0.27	0.97
Proto-Pasta (before activation) Linear range: 50-300 µg/mL	**50 µg/mL**	0.42	2.93	2.44	0.08
	100 µg/mL	0.70	0.33	0.45	2.31
	200 µg/mL	1.28	0.90	2.30	1.52
Proto-Pasta (after activation) Linear range: 20-300 µg/mL	**50 µg/mL**	1.17	2.79	2.15	0.45
	100 µg/mL	2.46	1.23	1.22	3.74
	200 µg/mL	0.85	2.03	0.85	0.75
Black Magic (before pre-treatment) Linear range: 50-300 µg/mL	**50 µg/mL**	4.62	7.19	6.54	4.07
	100 µg/mL	5.53	4.10	7.47	5.43
	200 µg/mL	9.65	4.63	4.48	7.90
Black Magic (after pre-treatment) Linear range: 50-300 µg/mL	**50 µg/mL**	4.84	4.12	5.82	8.36
	100 µg/mL	11.27	7.52	6.23	12.93
	200 µg/mL	10.54	9.64	4.69	11.66
Graphite sheet Linear range: 10-300 µg/mL	**10 µg/mL**	0.76	0.48	0.86	1.89
	100 µg/mL	0.41	1.72	0.95	1.48
	200 µg/mL	1.93	1.63	0.60	0.16
NG/PLA AM-electrode Linear range: 20-300 µg/mL	**20 µg/mL**	3.07	0.95	2.67	2.93
	100 µg/mL	0.21	2.40	2.27	2.93

	200 µg/mL	2.08	1.98	2.65	0.03

Figure S1. The shape of the graphite screen-printed macroelectrode (SPE) inserted into the AM/3D-printed LC-AD flow cell.

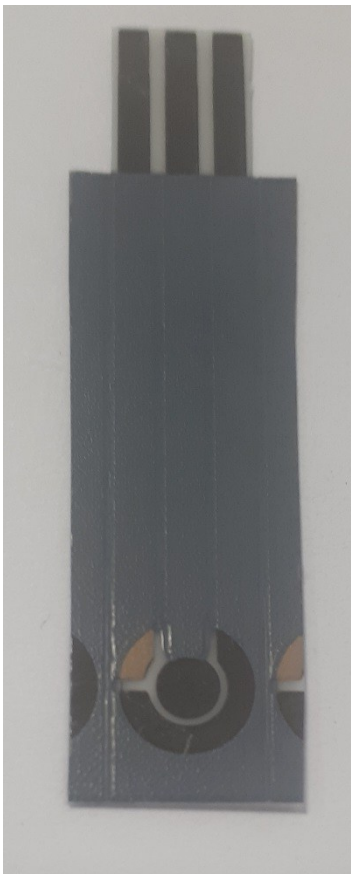


Figure S2. Cyclic voltammograms of 100 µg mL^{-1} of each of 25F-NBOMe, 25C-NBOMe, 25B-NBOMe and 25I-NBOMe in 0.04 M B-R buffer (pH 7.0) using SPEs. Scan rate: 50 mV s^{-1} *vs.* Ag/AgCl reference electrode. **(I)** First NBOMes' oxidation peak; **(II)** second NBOMes' oxidation peak[21].

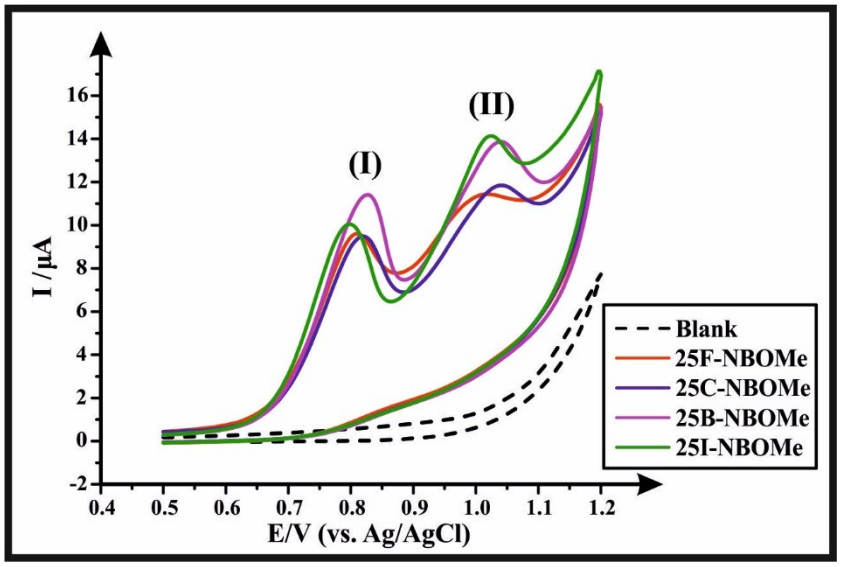
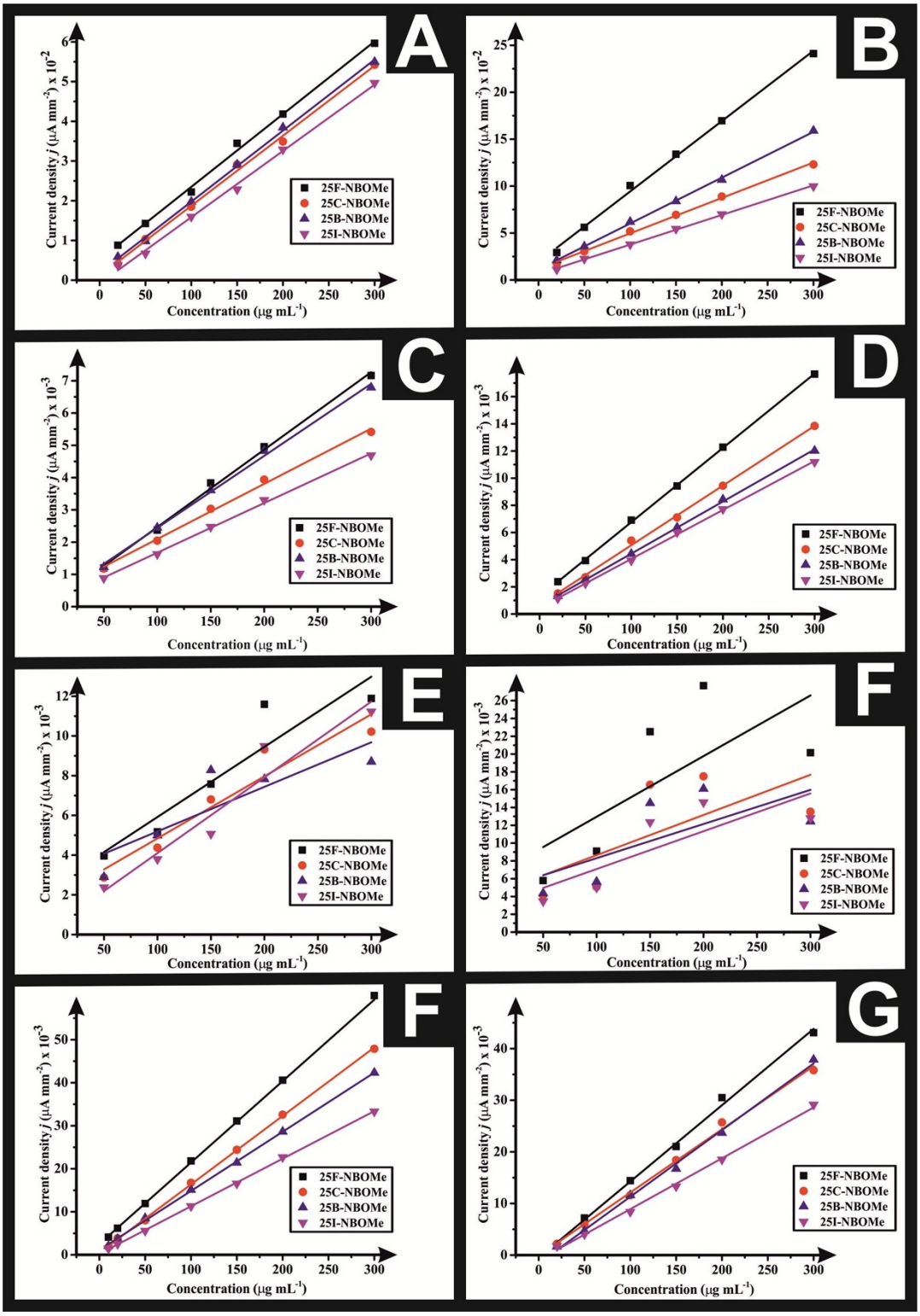


Figure S3: Calibration plots of the four NBOMe derivatives employing the AM/3D printed flow cell using the following working substrates: (**A**) graphite screen printed macroelectrodes (SPEs), (**B**) SPEs utilising the commercial flow cell; (**C**) Proto-Pasta (before activation); **(D)** Proto-Pasta (after activation); **(E)** Black Magic (before pretreatment); **(F)** Black Magic (after pretreatment); **(G)** graphite sheet; **(G)** NG/PLA AM- electrode. Nickel wire and Proto Pasta filament covered with silver ink acted as the counter and reference electrodes, respectively, for all the tested working substrates except the SPEs. Note that the current density, *j*, in case of the SPEs is multiplied by 10^{-2} while it is multiplied by a factor of 10^{-3} in the rest of the tested electrodes.



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