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Sensitive screening for drugs of abuse in human urine using single quadrupole GC-MS following a simple solid phase extraction

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Keywords

SPE, urine, GC-MS, advanced electron ionization, AEI, single quadrupole, deconvolution, screening, drugs of abuse, forensic toxicology

Goal

To assess a fast, robust, and reliable method of screening for drugs of abuse in urine samples in a routine and high-throughput forensic laboratory.

Introduction

In many forensic investigations there is a requirement to analyze drugs of abuse (DoA) in human bodily fluids. In many cases, a reliable and affordable methodology is needed given the high number of samples that must be investigated and the average price per sample the laboratories can charge. One of the most important requirements for this application is a sensitive method, which can be used to selectively detect a large number of drug groups, such as opiates, amphetamines, synthetic cannabinoids, and others, in one single method at very low levels. This is a challenging task for any laboratory as in addition to being sensitive, the method requires a simple, cost-effective sample preparation and a robust and easy to implement GC-MS method.

The matrix screened is mainly urine and the drugs of abuse can be detected for approximately one week after last use. Urine samples are biologically complex, reflecting the state of the metabolism and life style habits of the subjects. Consequently, many drug substances and their metabolites will be present at quite low levels in the sample, making it challenging to detect them

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selectively and sensitively. Therefore, additional sample preparation steps such as solid phase extraction (SPE) are useful to reduce the chemical background and to concentrate the analytes of interest. GC-MS operated in electron ionization (EI) mode is very often used for this application, since the spectra generated are library searchable against existing commercial and private libraries.[1]

In this application note a complete solution—from the urine samples to the results—is presented. This includes a detailed description of the sample preparation SPE protocol together with the GC-MS parameters and the results obtained in several urine samples using automated spectral deconvolution software. Peak deconvolution is a key tool for this application, given the complexity of the matrix samples analyzed that will make manual compound identification a very laborious task.

Experimental

Sample preparation

The drugs of abuse excreted in urine are in the form of glucoronidate conjugates. Therefore, beta-glucuronidase is used for enzymatic hydrolysis. This enzyme is commonly used during sample preparation to cleave off glucuronides and sulfate esters prior to GC-MS analysis.

The urine samples were subjected to a solid phase extraction procedure:

1. 30 µL β-glucuronidase (Merck 5000 I.U.) were added to 3 mL of urine and incubated for 60 min at 56 °C.

2. The Thermo Scientific™ HyperSep™ Verify CX cartridge, 6 mL/200 mg, was conditioned with 3 mL MeOH followed by 3 mL 0.1% formic acid.

3. Urine was mixed with 3 mL of 2M acetate buffer, pH 4.8. Urine was checked and adjusted, where necessary, for accurate pH.

4. The sample was added to the HyperSep Verify CX cartridge and a slight vacuum was applied to achieve, for example, approximately one drop per second elution rate.

5. Interference elution was done with a mixture of 1 mL water + 0.1% formic acid, total volume 3 mL, followed by a mixture of 1 mL MeOH/water 50:50 + 0.1% formic acid, total volume 3 mL.

6. The cartridge was dried after interference elution with strong vacuum.

7. Elution was performed with a mixture of methanol and 5% ammonia solution (95:5), pH 9, two times 0.5 mL.

8. The sample was evaporated under nitrogen until dryness at 65 °C.

9. The sample was reconstituted with 50 µL MeOH and placed in 50 µL MS certified vials.

The vials were subsequently centrifuged for precipitating the particles before putting them in the autosampler.

Consumables

- Thermo Scientific™ HyperSep™ Verify CX Cartridges, 200 mg/3 mL; 50 pack (P/N 60108-777)
- 24-port Vacuum Manifold (P/N 60104-233)
- Vacuum Pump (P/N 60104-233)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT100)
- Analyte Elution 4 mL Vial
- Thermo Scientific™ Reacti-Vap™ Evaporator (P/N TS-18826)
- Thermo Scientific™ SureStop™ MS Certified Vials, 100 µL reservoir (P/N MSCERT5000-36LVW)
- Thermo Scientific™ Marathon™ Injection Port Septa (P/N 313P3240)
- Thermo Scientific™ LinerGOLD™ GC Liners (P/N 453A-1255-UI)
- Thermo Scientific™ TRACE™ TR-DoA 35MS column, 15 m, 0.25 mm ID, 0.25 µm (P/N 26AF130P)

GC-MS parameters

Compound separation and detection was achieved using a Thermo Scientific™ TRACE™ 1310 GC coupled with a Thermo Scientific™ ISQ™ 7000 mass spectrometer with the Advanced Electron Ionization (AEI) source that offers unparalleled sensitivity. Sample introduction was performed using a Thermo Scientific™ TriPlus™ 100 LS autosampler, injecting 1 µL into the Thermo Scientific™ Instant Connect Split/Splitless (SSL) injector module. Tables 1 and 2 list the method parameters.

Table 1. GC oven and injection method

Oven Method	
Initial temperature:	70 °C
Initial hold time:	0.5 min
Ramp 1 rate:	22 °C/min
Ramp 1 final temperature:	320 °C
Ramp 1 hold time:	2 min
S/SL Method	
S/SL mode:	Splitless with Surge
Temperature:	280 °C
Splitless time:	1 min
Split flow:	20 mL/min
Surge pressure:	172 kPa
Surge duration:	1 min
Purge flow:	5 mL/min
Carrier mode:	Constant Flow
Carrier flow:	1.5 mL/min
Vacuum compensation:	On

Table 2. ISQ 7000 GC-MS system parameters

MS transfer line temperature:	250 °C
Ion source temperature:	270 °C
Ionization mode:	EI
Acquisition start time (or solvent delay):	1.5 min
Start mass:	50 amu
End mass:	550 amu
Scan time:	0.2 s

Data processing

Data was acquired in full-scan mode and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. The data was subsequently sent to AnalyzerPro® software to perform automated chromatographic deconvolution and peak detection, followed by library searching for putative compound identification. For this application, the freely downloadable SWGDRUG library was used, together with the NIST, PMW and the LVR in-house developed drugs of abuse library. AnalyzerPro software allows for searching on multiple spectral libraries at the same time and can perform the reprocessing in a batch format. The software can detect a large number of compounds present in a sample, including more challenging ones such as amphetamine and small metabolites of new psychoactive substances.[2-5] Other capabilities of the software include making a customized layout for a specific compound using the extracted ion chromatograms and adding spectra to a target library. In addition, using retention time indexing is possible for more accurate putative identification of isomeric compounds and a tentative quantitation is also possible by using internal standards as reference peaks. However, for these samples, only the deconvolution in combination with unknown screening was used.

Results and discussion

Over the course of several weeks, over 700 urine samples were analyzed following the sample preparation and workflow described. Example results were selected to demonstrate the capability of this methodology to analyze various challenging drug metabolites and some typical profiles of urine samples. Ten urine samples (A to J) were found to be the most interesting, and data for these samples is summarized in Table 3.

From the analyses of urine profiles, the behavior of the subjects could be inferred. For instance, urine sample D appears to show a quick ingestion of all available drugs before the forensic investigation started; whereas urine sample J could present a subject going to multiple doctors trying to get a prescription for pregabalin, which in high quantities offers a feeling of being high. Sample H potentially demonstrates a subject taking medication to cope with withdrawal symptoms. Interestingly, in almost all the urine samples, markers for smoking such as cotinine and nicotine were found.

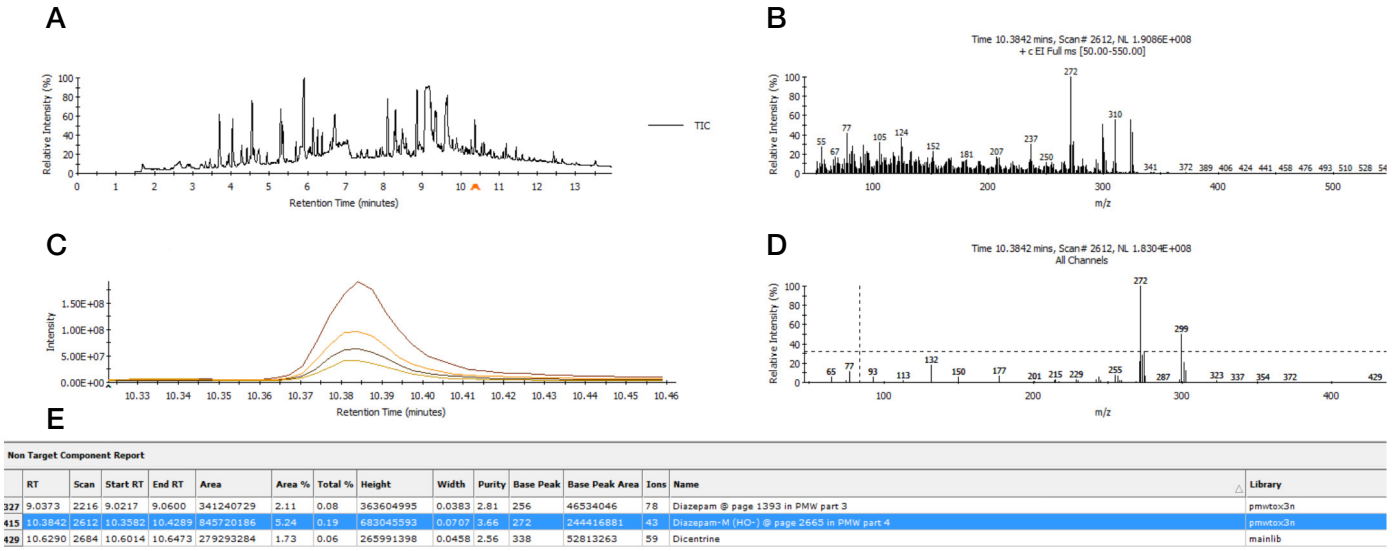
Table 3 (Part 1). Results of drugs screening in samples A–D. Putatively identified compound name, chemical class, as well as retention time and library search scores (SI, RSI, and confidence), are shown. Confidence level is a spectral matching value derived from the NIST library search results.

Urine	Compounds	Class	RT	SI	RSI	Confidence
A	Clonazepam metabolite	Benzodiazepine	10.6	703	824	73.9
	Anhydro Ecgonine methyl ester	Opiates	4.1	689	796	72.2
	Meconin	Opiates	5.9	856	878	86.3
	Methadon metabolite EDDP	Opiates	7.4	746	776	75.5
B	Clonazepam metabolite	Benzodiazepine	10.6	762	813	77.7
	Ibuprofen	NSAID	5.3	818	846	82.6
	Methadon	Opiates	7.8	658	683	66.6
	methadon metabolite EDDP	Opiates	7.3	804	840	81.5
	Nordazepam	Benzodiazepine	9.1	732	784	74.8
C	Benzoyl ecgonine	Opiates	9.6	709	781	73.1
	Codeine	Opiates	8.8	741	756	74.6
	methadon	Opiates	7.8	831	867	84.2
	methadon metabolite EDDP	Opiates	7.3	883	924	89.5
	Morphine	Opiates	9.1	870	882	87.4
	Papaverine	Opiates	10.6	654	689	66.5
	Paracetamol	analgesic	6.7	614	655	62.6
D	acetylcodeine	Opiates	9.3	612	694	63.7
	Anhydro Ecgonine methyl ester	Opiates	4.0	911	916	91.3
	Benzoyl ecgonine	Opiates	9.6	848	851	84.9
	Coca ethylene	Opiates	8.3	743	821	76.6
	Codeine	Opiates	8.9	816	819	81.7
	Diazepam M	Benzodiazepine	10.4	710	732	71.7
	ecgonine methyl ester	Opiates	4.5	895	900	89.7
	Ibuprofen	NSAID	5.3	646	687	65.8
	Meconin	Opiates	5.9	899	904	90.1
	Methadon	Opiates	7.8	631	736	66.3
	Methadon metabolite EDDP	Opiates	7.3	697	782	72.3
	Mirtazapine	Antidepressant	9.8	741	761	74.7
	Nordazepam	Benzodiazepine	8.8	789	901	82.3
	Noscapine	Opiates	10.0	855	864	85.8
	Oxazepam	Benzodiazepine	7.5	644	769	68.2
	Papaverine	Opiates	10.4	733	855	77.0
	Temazepam	Benzodiazepine	8.4	622	632	62.5
	Temazepam artefact 1	Benzodiazepine	8.5	845	901	86.2
	Temazepam artefact 2	Benzodiazepine	9.9	588	765	64.1
	Temazepam artefact 1	Benzodiazepine	8.5	845	901	86.2
	Temazepam artefact 2	Benzodiazepine	9.9	588	765	64.1

Table 3 (Part 2). Results of drugs screening in samples E–J. Putatively identified compound name, chemical class, as well as retention time and library search scores (SI, RSI, and confidence), are shown. Confidence level is a spectral matching value derived from the NIST library search results.

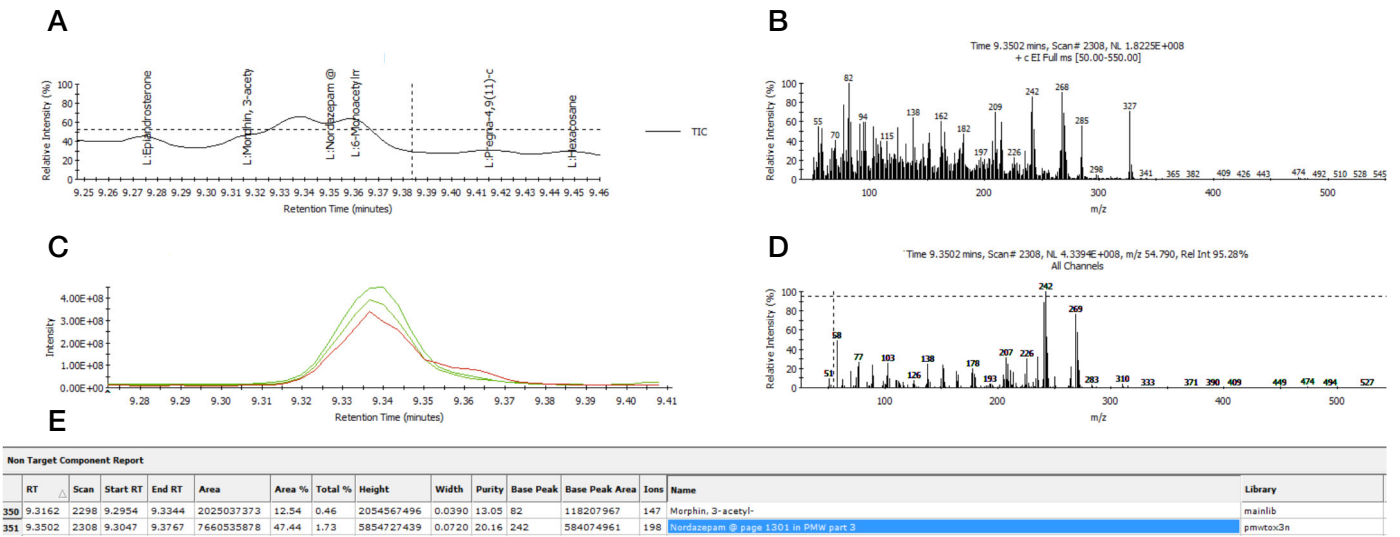
Urine	Compounds	Class	RT	SI	RSI	Confidence
E	Benzoyl ecgonine	Opiates	9.7	832	852	83.8
	Bromazepam	Benzodiazepine	9.8	740	779	75.2
	Carbamazepine	Benzodiazepine	8.6	574	619	58.8
	Cocaine	Opiates	8.1	584	792	64.6
	Codeine	Opiates	8.7	758	760	75.9
	Diazepam M	Benzodiazepine	9.0	685	751	70.5
	Methylecgonine	Opiates	4.5	868	942	89.0
	Ibuprofen	NSAID	5.6	643	704	66.1
	Levomepromazine-M (nor-HO$^-$)	Neuroleptic	10.1	605	669	62.4
	Levomepromazine-M/A (sulfoxide)	Neuroleptic	10.7	675	701	68.3
	Meconin	Opiates	5.9	925	926	92.5
	Methadon metabolite EDDP	Opiates	7.3	664	753	69.1
	Morphine	Opiates	9.1	619	619	61.9
	Oxazepam	Benzodiazepine	7.6	735	780	74.9
	Papaverine	Opiates	10.4	545	607	56.4
	Quetiapine	Neuroleptic	11.7	876	882	87.8
F	Amphetamine	Amphetamines	2.1	752	829	77.5
	Codeine	Opiates	8.9	928	930	92.9
	Heroin-M (6-acetyl-morphine)	Opiates	9.4	740	835	76.9
	Hydrocotarnine	Opiates	6.3	793	872	81.7
	Morphine	Opiates	9.1	910	918	91.2
G	Chlorprothixene	antipsychotic	10.6	856	861	85.8
	Carbamazepine	Benzodiazepine	9.9	775	826	79.0
	oxycodon	Opiates	10.7	883	890	88.5
	Tilidine metabolite	Opiates	7.4	825	871	83.9
	Tilidine-M (bis-nor$^-$)	Opiates	7.3	689	756	70.9
	Tilidine-M (bis-nor-HO$^-$)	Opiates	8.3	588	636	60.2
H	Diphenhydramine	Antihistamine	9.3	680	696	68.5
	Venlafaxine	Antidepressant	8.5	621	738	65.6
I	Amphetamine	Amphetamines	2.1	760	771	76.3
	Pipamperone	Neuroleptic	11.1	898	908	90.1
J	Benzoyl ecgonine	Opiates	9.6	687	860	73.9
	Cocaine	Opiates	8.1	920	927	92.2
	Codeine	Opiates	8.8	878	878	87.8
	ecgonine methyl ester	Opiates	4.5	851	852	85.1
	Levomeprazine	Neuroleptic	10.6	763	787	77.0
	Meconine	Opiates	5.9	928	940	93.2
	Methadon	Opiates	7.8	881	893	88.5
	Methadon metabolite EDDP	Opiates	7.3	917	955	92.8
	Mirtazapine	Antihistamine	8.3	920	927	92.2
	Morphine	Opiates	9.2	667	668	66.7
	Pregabaline	Anticonvulsant	4.3	731	750	73.7

Figures 1 to 3 provide detailed views of some of the more challenging metabolites.



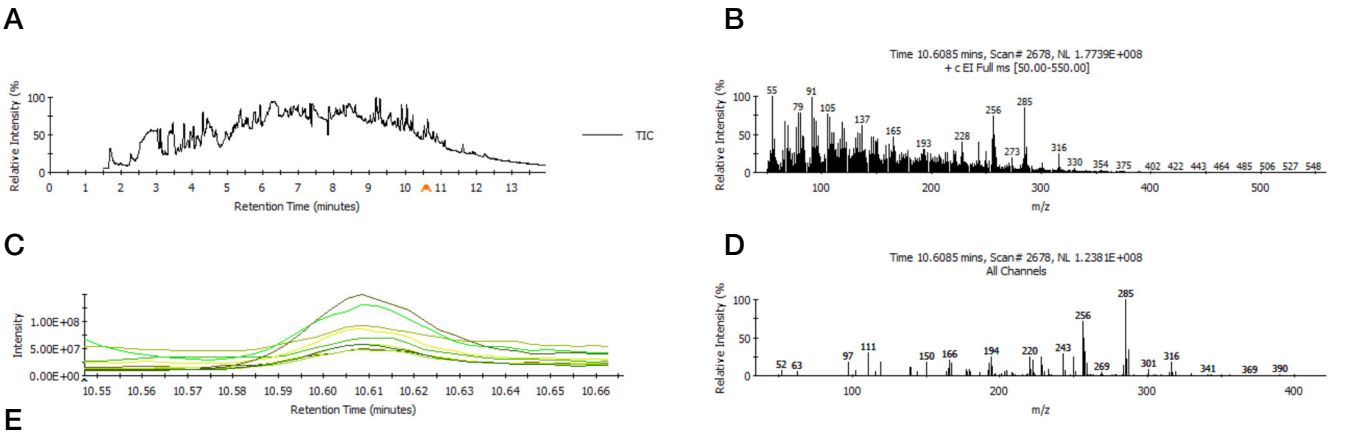
	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name	Library
327	9.0373	2216	9.0217	9.0600	341240729	2.11	0.08	363604995	0.0383	2.81	256	46534046	78	Diazepam @ page 1393 in PMW part 3	pmwtox3n
415	10.3842	2612	10.3582	10.4289	845720186	5.24	0.19	683045593	0.0707	3.66	272	244416881	43	Diazepam-M (HO-) @ page 2665 in PMW part 4	pmwtox3n
429	10.6290	2684	10.6014	10.6473	279293284	1.73	0.06	265991398	0.0458	2.56	338	52813263	59	Dicentrine	mainlib

Figure 1. (A) Chromatogram of urine sample D in full-scan mode, (B) raw spectrum at the retention time of diazepam metabolite, (C) extracted ions of the analyte, (D) deconvoluted spectrum, (E) name and RT of the putatively identified compound, in this case diazepam metabolite



	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name	Library
350	9.3162	2298	9.2954	9.3344	2025037373	12.54	0.46	2054567496	0.0390	13.05	82	118207967	147	Morphin, 3-acetyl-	mainlib
351	9.3502	2308	9.3047	9.3767	7660535878	47.44	1.73	5854727439	0.0720	20.16	242	584074961	198	Nordazepam @ page 1301 in PMW part 3	pmwtox3n

Figure 2. (A) Chromatogram of urine sample D in full-scan mode, zoomed in at RT of nordazepam, showing its co-elution with other drugs and compounds, (B) raw spectrum at the retention time of nordazepam metabolite, (C) extracted ions of the analyte, (D) deconvoluted spectrum, (E) name and RT of the putatively identified compound



Non Target Component Report

	RT	Scan	Start RT	End RT	Area	Area %	Total %	Height	Width	Purity	Base Peak	Base Peak Area	Ions	Name
499	10.6085	2678	10.5809	10.6306	1433694879	9.74	0.21	1130626909	0.0498	4.78	285	212534755	117	Clonazepam-M (amino-) page 1402 in PMW part 3

Figure 3. (A) Chromatogram of urine sample A in full-scan mode, (B) raw spectrum at the retention time of the clonazepam metabolite, (C) extracted ions of the analyte, (D) deconvoluted spectrum, (E) name and RT of the putatively identified compound, in this case clonazepam

Observations on GC-MS maintenance

For a routine laboratory that operates continuously, method robustness is a key factor. To monitor the sensitivity of the system, morphine was used as a marker. Due to its polarity, it is the perfect analyte to monitor the liner as well as the condition of the ion source. After every 10 injections of urine samples, a standard was analyzed to assess and document the condition of the system. The setup of the method allowed time to run 60 samples per day on average.

For this workflow, the LinerGOLD liner needs only to be replaced every 50 injections. Liner replacement is tool free and quick and easy on the Instant Connect Split/Splitless injector with no compromise on the MS vacuum. The robustness of the source allowed for over 700 injections without cleaning, at which time the analytical column is normally due to be replaced. The SmartTune of the instrument was performed daily for monitoring the air/water background and the response of the system. The first re-tuning of the MS was needed only after approximately 300 urine injections.

Conclusions

The data obtained from the experiments performed demonstrate that the ISQ 7000 GC-MS system with AEI source, in combination with the HyperSep Verify CX cartridge for sample preparation and Chromeleon CDS for data processing, provided a single quick, cost-effective, and robust method for the general unknown screening. This method can be used for routine analysis of drugs of abuse in urine with a sample throughput of >12,000 samples/year.

The concentration factor of 60 gained through sample preparation and the enhanced sensitivity of the ion source made it possible to reach low detection limits, which are much more sensitive than the results gained in immunological testing. This is a big advantage, especially for new psychoactive substances.

Chromatographic deconvolution is an absolutely invaluable tool for this kind of analysis, where hundreds of substances are present in the chromatograms. Manual data interrogation would be time consuming and would require a highly experienced analyst.

The ISQ 7000 GC-MS system in combination with Chromeleon CDS software and AnalyzerPro software provides the perfect workflow for the general unknown screening for drugs of abuse.

References

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