Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method

Using Scheduled MRM™ Algorithm on the SCIEX QTRAP® 6500+ LC-MS/MS System

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The ability to accurately identify the presence of a variety of drugs and drug metabolites in biological specimens is a critical aspect to any forensic and clinical toxicology investigation as it provides a comprehensive picture of past drug exposure towards xenobiotics, a history of the non-endogenous substances in the human body. Detection of these substances can be performed in several biological matrices including blood, urine, hair, sweat and saliva. Although urine and blood testing are the most common forms of drug testing, hair analysis has gained considerable attention over the years as a method enabling the determination of recent past drug use as well as the long term drug use through segmental analysis. Additional benefits of hair testing include the non-invasive nature of sample collection and the ease of sample storage and transportation. These advantages considerably minimize the risk of sample alteration and degradation over time as well as the risk of exposure to biohazards. As a result, these attribues are driving the wide-spread adoption of hair testing to address a wide range of challenges including postmortem analysis, DUID screening, therapeutic drug monitoring and drug-facilitated assault (DFA) investigations, all while providing a broader picture of past drug consumption and abuse with a longer detection window (months to years).

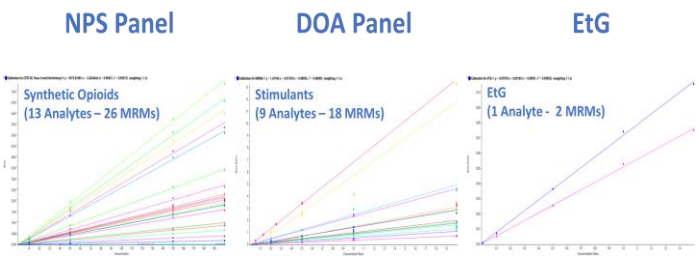


Figure 1: High linearity demonstrated across all the analytes included in the three panels. Representative calibration curves from each of the three panels showing excellent linear response across the calibration series, demonstrating the wide applicability of the sample preparation procedure to a large variety of drug and drug metabolite chemistries. The three separate workflows showed excellent linearity across their respective calibration ranges, resulting in R^2 values > 0.98 for all the analytes.

A comprehensive workflow for the detection of a wide range of drugs and drug metabolites in hair was successfully developed. The combination of an easily implemented sample extraction procedure with the sensitivity of the SCIEX QTRAP 6500+ System has enabled accurate identification and sensitive quantification of a wide range of chemically-diverse analytes: (panel 1) Novel Psychoactive Substances (NPS), (panel 2) Drugs Of Abuse (DOA) and (panel 3) EtG, a direct alcohol metabolite used as an indicator of alcohol consumption.

Key features of the QTRAP 6500+ LC-MS/MS System for sensitive multi-drug panel detection in hair samples

- IonDrive™ Technology on the QTRAP 6500+ System enabled optimum ionization efficiency and ion sampling, resulting in high detection sensitivity of the drugs and drug metabolites in the three panels (high pg/mg to low ng/mg)
- 14-step sample preparation procedure enabled extraction of of a wide array of chemically-diverse drugs and drug metabolites from real hair samples
- Three different LC separation methods resulted in near baseline separation of all drugs and drug metabolites present in each of the respective panels
- Method was applied to real hair samples collected from subjects who were suspected of past non-medical NPS use, recent DOA use or alcohol consumption
- Workflow allowed accurate identification and sensitive quantification of sub pg/mg detection limits of drugs and drug metabolites in these real hair samples

Methods

Hair sample preparation: ~50 mg of head hair were cut into small segments and twice-washed with methanol then diethyl ether (5 mL of solvent, votexed for 2 minutes). The solvent washes were removed following each votexing step. Following the washing steps, the hair samples were dried for 15 minutes at 60˚C under a gentle nitrogen flow. 25 mg of hair were weighed and placed in a glass tube. The resulting hair samples were spiked with various drug calibrator mixtures and internal standard mixes (when applicable). 500 µL of aqueous extracting solution was added to the spiked hair samples, then gently vortexed and the resulting solution was centrifuged at 5,000 rpm for 1 minute. The resulting mixture was incubated for 60 minutes at 100˚C. Following the incubation step, the liquid extract was transferred to a new tube and centrifuged at 5,000 rpm for 5 minutes. The organic phase was transferred to a sample vial for analysis. A summary of the hair samples preparation and extraction procedure is shown in Figure 2.

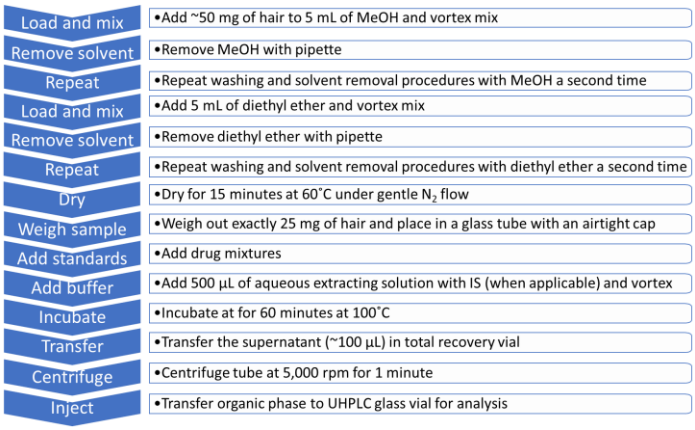


Figure 2. Hair samples preparation and extraction workflow. A fast, 14-step extraction procedure was used for extracting the drugs and drug metabolites included in the NPS, DOA and EtG panels from real hair samples for MS analysis.

NPS calibrator mixture preparation: A total of 77 compounds were included in the NPS panel. They consisted of 32 synthetic cannabinoids, 8 phenethylamines, 13 synethtic opioids (fentanyl analogs), 10 synthetic opioid metabolites, 11 tryptamines, 1 cathinone, 1 piperidine and 1 amino acid (endogenous serotonin precursor). A 0.25 µg/mL stock standard solution mixture containing all the NPS was prepared in methanol and used for initial method development. This solution was used to pepare a series of 4 calibrator solutions that were used to spike the 25 mg of hair samples, resulting in final NPS concentrations ranging from 10 to 200 pg/mg. Table 1 summarizes the NPS used in this panel and lists them by drug class.

DOA calibrator mixture preparation: A total of 23 compounds and 20 internal standards (IS) were included in the DOA panel. The DOA panel consisted of 6 opioids, 9 stimulants, 4 anti-depressants, 3 cannabinoids and 1 benzodiazepine. A 5 µg/mL stock standard solution mixture containing all the DOA and a 200 pg/mL IS solution were both prepared in methanol. A series of 4 calibrator solutions containing the 23 compounds were used to spike 25 mg of hair samples. These calibrator solutions were mixed with the aqueous extracting solution that was previously spiked with the internal standard (IS) solution, resulting in final DOA concentrations ranging from 0.05 to 2 ng/mg and a final IS concentration in each sample of 1 ng/mg, respectively. The full list of the DOA used in this panel is summarized in Table 2.

EtG calibrator mixture preparation: A 500 ng/mL solution of EtG was prepared in methanol. This solution was used to pepare a series of 4 calibrator solutions that were used to spike 25 mg of hair samples. These calibrator solutions were mixed with the aqueous extracting solution that was previously spiked with the internal standard (IS) solution, resulting in final EtG concentrations ranging from 20 to 300 pg/mg and a final IS (EtG-d5) concentration in each sample of 1 ng/mg, respectively.

Liquid chromatography: UHPLC separations were performed on a Phenomenex Synergi 2.5 µm Hydro-RP column (50 x 3 mm, 2.5 µm, P/N 00D-4387-Y0) at 50 ºC on the ExionLC™ AC System (SCIEX). Mobile phases used consisted of water, acetonitrile and modifiers. Three separate gradients were used for each of the LC methods, resulting in total run times of 18 min for the NPS panel (LC flow rate of 0.5 mL/min, 4 µL injection volume), 8 min for the DOA panel (LC flow rate of 0.5 mL/min, 2 µL injection volume) and 4 min for the EtG experiment (LC flow rate of 0.4 mL/min, 3 µL injection volume), respectively.

Mass spectrometry: MS data was collected in positive electrospray ionization (ESI) mode on the SCIEX QTRAP 6500+ System equipped with an IonDrive Turbo V Ion Source. Three separate acquisition methods were created using the Scheduled MRM Algorithm in Analyst® Software 1.7.1 to ensure acquisition of an adequate amount of data points across the LC peak for high quality, quantifiable data. The acquisition methods consisted of 156 MRM transitions for the NPS panel, 71 MRM transitions for the DOA panel and 3 MRM transitions for the EtG experiment, respectively.

Data analysis: Data processing was performed in SCIEX OS Software 1.5. Detection and integration of the peaks from the background was achieved within the viewing window using the AutoPeak Algorithm. Quantitative analysis was performed in the Analytics module of the software where calibration curves and concentration calculations were generated.

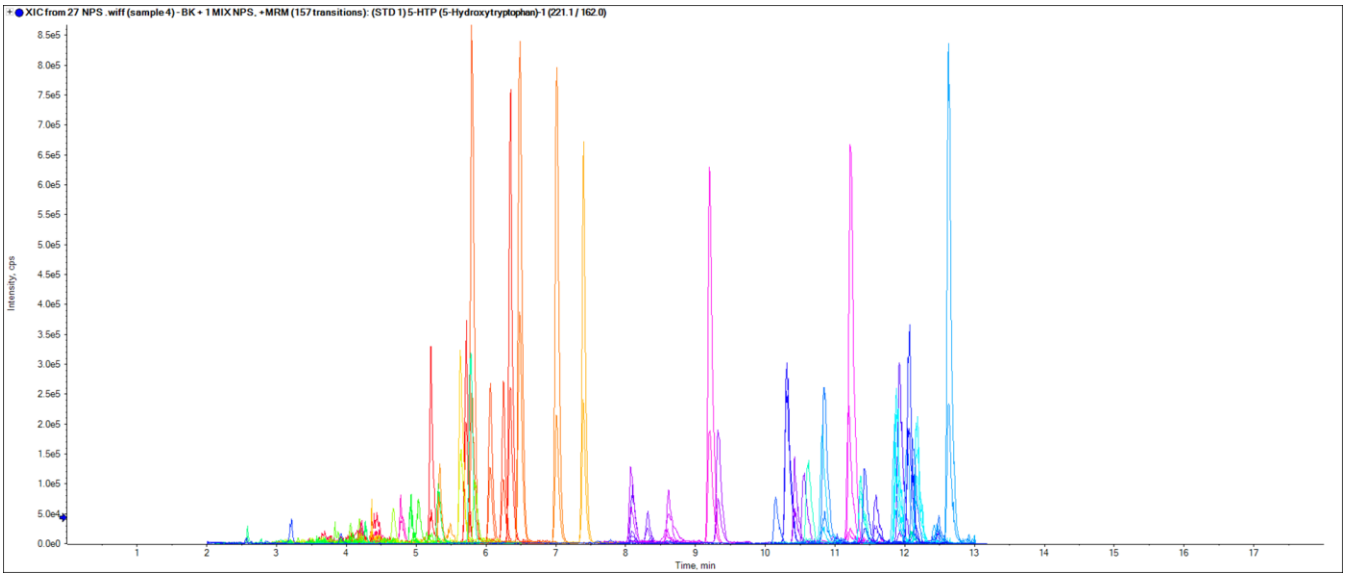


Figure 3: Chromatographic profile of the Novel Synthetic SubstancesOpioid (NPS) panel by LC-MS analysis. Extracted Ion Chromatograms (XICs) resulting from the optimized LC separation conditions which enabled near baseline separation of the 77 NPS included in the panel. The combination of the 18-minute gradient and 0.5 mL/min flow rate was necessary to separate the closely related fentanyl analogs and other structurally-related NPS in the panel.

Novel Psychoactive Substances (NPS) results

Control head hair samples were spiked with the 77 compounds of the NPS panel at various concentrations, resulting in final NPS concentrations ranging from 10 to 200 pg/mg. These standard hair mixtures were extracted using the aforementioned procedure and injected to build a data processing method.

Figure 3 shows the extracted ion (XIC) traces for the 77 NPS in a control head hair sample at a final NPS concentration of 10 pg/mg. The 18 minute long LC run time was necessary to achieve near baseline separation of all the NPS and to resolve the structurally-related and isobaric fentanyl analogs and synthetic cannabinoids included in the panel.

Calibration curves were generated to evaluate the linearity and quantification performance of the NPS method. Figure 4 shows the results from the calibration series performed for each drug class and includes representative calibration curves from 10 to 200 pg/mg for the 32 synethtic cannabinoids (A), 8 phenethylamine (B), 13 synthetic opioids (C), 10 synthetic opioid metabolites (D), 11 tryptamines (E) and one methylphenidate (F). Two MRM transitions were used to evaluate the quantitative response of each analyte. Overall, the calibration curves demonstrated excellent linearity for all sample types, with R^2 values > 0.98 for all the analytes included in the panel.

The robustness of the NPS screening method was further investigated by analyzing 10 real hair case samples from subjects who were suspected of past non-medical NPS use. These case samples were prepared using the aforementioned procedures and run using the NPS screening method. Figure 5 shows a screenshot of the SCIEX OS Software Results Table showing the NPS positively identified in a real hair case sample along with their extracted ion chromatograms (XICs). The results table lists the drugs detected along with their calculated concentration. The two synthetic cannabinoids JWH-122 and AM-2201 were detected in case sample number 3 at concentration of 111.8 and 11.72 pg/mg, respectively.

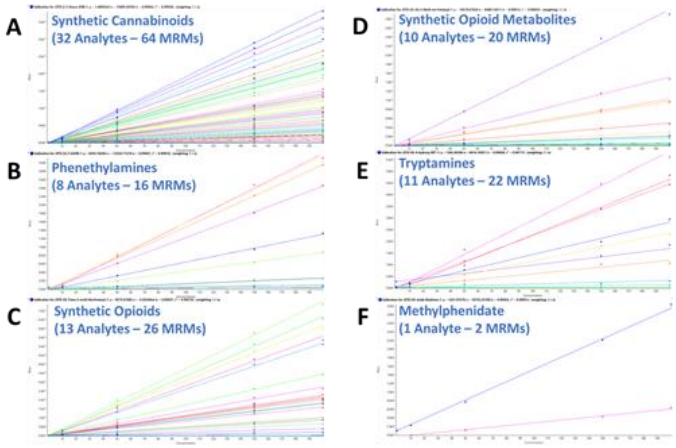


Figure 4. Representative calibration curves resulting from the calibration series of the NPS organized by drug class from 10 to 200 pg/mg. High linearity (R^2 values > 0.98) was observed across all the analytes in the NPS panel. Representative calibration curves from 10 to 200 pg/mg were created by drug class and include synthetic cannabinoids (A) phenethylamines (B), synthetic opioids (C), synthetic opioid metabolites (D), tryptamines (E), methylphenidate (F), as well as a cathinone, a piperidine and an amino acid (not included in the figure).

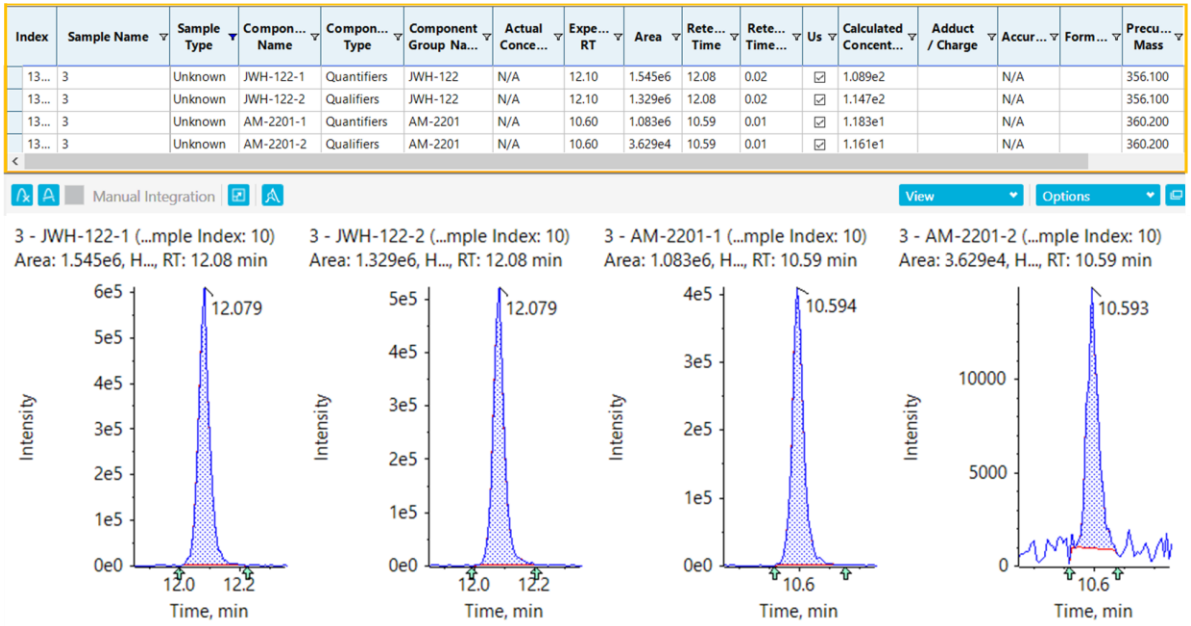


Figure 5: Robust NPS screening method enables positive identification of two synthetic cannabinoids in a real hair case sample. SCIEX OS Software results table showing positive detection of JWH-122 and AM-2201 in case sample number 3 at concentration of 111.8 and 11.72 pg/mg, respectively (top panel). XICs showing integration of the peaks for each MRM transition of JWH-122 and AM-2201 (bottom panel). Efficient review and easy visualization of the results was enabled by using the filtering options in SCIEX OS Software.

Drugs of Abuse (DOA) results

A second set of control head hair samples was spiked with the 23 analytes of the DOA panel at various concentrations. These standard hair mixtures were mixed with the aqueous extracting solution that was previously spiked with the 20 DOA internal standards (IS), resulting in final DOA concentrations ranging from 0.05 to 2 ng/mg and a final IS concentration of 1 ng/mg, respectively. The resulting samples were extracted using the same sample preparation procedure and injected to build a data processing method. These samples were injected in duplicate.

Figure 6 shows the chromatographic profile of the 23 analytes included in the DOA panel at final DOA concentrations of 0.1 ng/mL. The 8-minute long gradient resulted in the near baseline separation of all the analytes in the DOA panels, as seen in the extracted ion chromatogram (XIC) traces (Figure 6).

The linearity of the DOA method was assessed by plotting the regression curves for the 23 analytes included in the panel. Figure 7 shows representative regression curves for each of the drug classes in the DOA panel and include the calibration series performed for 6 opioids (A), 9 stimulants (B), 4 anti-depressants (C) and three cannabinoids (D). Five levels of calibrators ranging from 0.05 to 2 ng/mg were used to evaluate the linearity of the method. The results demonstrated excellent correlation of the generated regression curves with R^2 values > 0.98 for all the analytes included in the DOA panel.

Figure 7 shows representative regression curves for each of the drug classes in the DOA panel and include the calibration series performed for 6 opioids (A), 9 stimulants (B), 4 anti-depressants (C) and three cannabinoids (D). Five levels of calibrators ranging from 0.05 to 2 ng/mg were used to evaluate the linearity of the method. The results demonstrated excellent correlation of the generated regression curves with R^2 values > 0.98 for all the analytes included in the DOA panel.

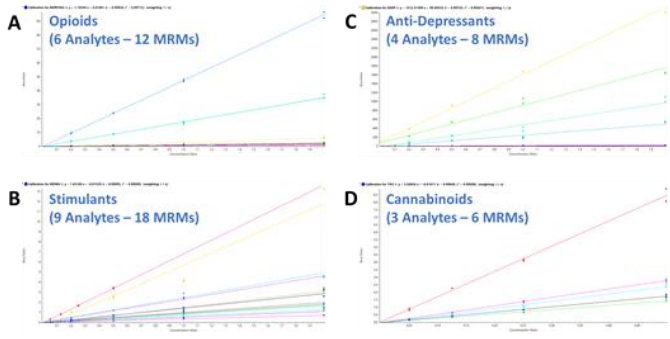


Figure 7. Representative calibration curves of four of the five drug classes making up the DOA panel. Excellent linearity (R^2 values > 0.98) across the calibration curves for the 23 analytes included in the DOA panel was observed for concentrations ranging from 0.05 to 2 ng/mg. Drug classes in the DOA panel include opioids, stimulants, anti-depressants, cannabinoids, as well as a benzodiazepine (not included in the figure).

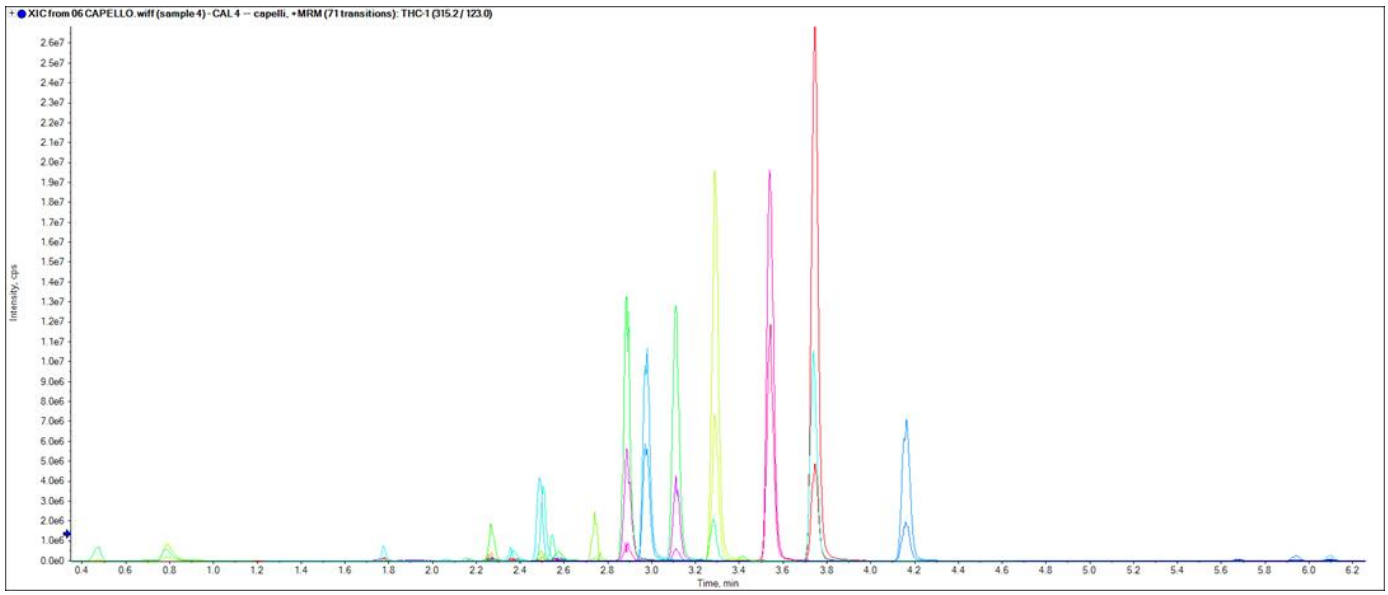


Figure 6: Chromatographic profile of the Drugs of Abuse (DOA) panel by LC-MS analysis. Extracted Ion Chromatograms (XICs) showing near basline separation of the resulting from near baseline separation of the 23 DOA included in the panel. A shorter (8 min) gradient in combination with a 0.5 mL/min flow rate enabled fast separation of the chemically-diverse drugs included in the DOA panel.

The developed DOA screening method was further evaluated by testing real hair samples from subjects who reported past drugs of abuse consumption. In this case study, 8 real hair samples were analyzed to evaluate the robustness of the developed DOA screening method. Figure 8 and 9 show the results table and the extracted ion chromatograms (XICs) of the drugs of abuse that were successfully detected in case sample 2 and 3.

Ketamine, oxycodone, tramadol and zolpidem were detected in tested head hair sample number 2 at concentration of 0.3852, 1602.5, 248.4 and 0.0421 ng/mg, respectively (Figure 8). THC, cocaine and two of its metabolites; benzoylecgonine and cocaethylene were detected in hair sample 3 at concentration of 0.2920, 0.06450, 0.07894 and 0.4568 ng/mg, respectively (Figure 9). SCIEX OS Software provided the ability to streamline the review of the results and efficienty summarize them in the results table portion of the Analytics module for efficient sample results review.

Index	Sample Na...	Sample T...	Component Name	Compon... Type	Component Group Na...	Actual Conce...	Expe... RT	Area	Rete... Time	Rete... Time...	Us	Calculated Concent...	Adduct / Charge	Accur...	Form...	Precu... Mass
800	2	Unknown	KETAMINA-1	Quantifiers	KETAMINA	N/A	2.60	6.997e6	2.60	0.00	☑	3.455e-1		N/A		238.200
801	2	Unknown	KETAMINA-2	Qualifiers	KETAMINA	N/A	2.60	6.324e6	2.60	0.00	☑	4.249e-1		N/A		238.200
814	2	Unknown	OXICODON...	Quantifiers	OXICODONE	N/A	2.26	1.241e7	2.30	0.04	☑	1.599e3		N/A		316.100
815	2	Unknown	OXICODON...	Qualifiers	OXICODONE	N/A	2.26	2.955e6	2.29	0.03	☑	1.606e3		N/A		316.100
822	2	Unknown	TRAMADOL...	Quantifiers	TRAMADOLO	N/A	2.78	1.269e7	2.78	0.00	☑	2.722e2		N/A		264.200
823	2	Unknown	TRAMADOL...	Qualifiers	TRAMADOLO	N/A	2.72	6.935e5	2.78	0.06	☑	2.246e2		N/A		264.200
844	2	Unknown	ZOLPIDEM-1	Quantifiers	ZOLPIDEM	N/A	2.93	3.739e7	3.01	0.08	☑	4.691e-2		N/A		308.400
845	2	Unknown	ZOLPIDEM-2	Qualifiers	ZOLPIDEM	N/A	2.93	2.062e7	3.01	0.08	☑	3.733e-2		N/A		308.400

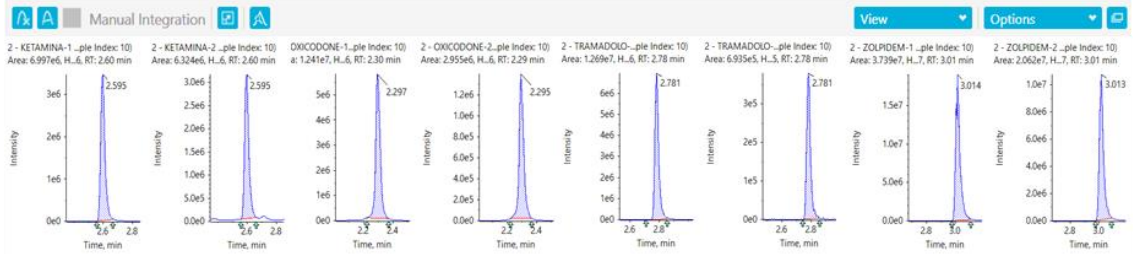
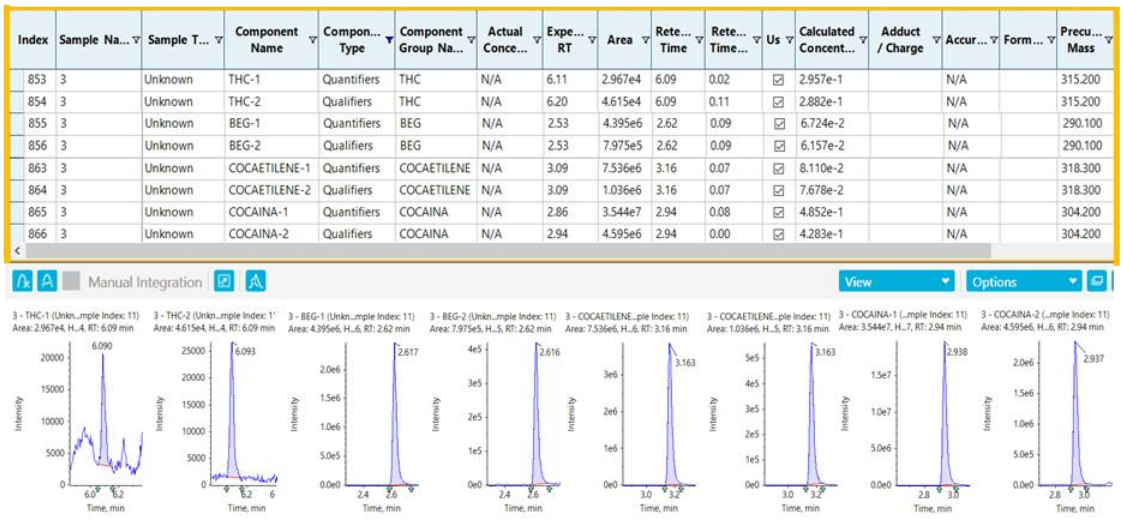


Figure 8: Optimized DOA screening method leads to positive identification of drugs of abuse in two real hair case samples. SCIEX OS Software results table showing positive identification of a number of drugs and drug metabolites in case sample 2 (top panel) along with XICs of the positively detected analytes (bottom panel). Ketamine, oxycodone, tramadol and zolpidem were positively detected in case sample number 2 at concentration of 0.3852, 1602.5, 248.4 and 0.0421 ng/mg, respectively.



Index	Sample Na...	Sample T...	Component Name	Compon... Type	Component Group Na...	Actual Conce...	Expe... RT	Area	Rete... Time	Rete... Time...	Us	Calculated Concent...	Adduct / Charge	Accur...	Form...	Precu... Mass
853	3	Unknown	THC-1	Quantifiers	THC	N/A	6.11	2.967e4	6.09	0.02	☑	2.957e-1		N/A		315.200
854	3	Unknown	THC-2	Qualifiers	THC	N/A	6.20	4.615e4	6.09	0.11	☑	2.882e-1		N/A		315.200
855	3	Unknown	BEG-1	Quantifiers	BEG	N/A	2.53	4.395e6	2.62	0.09	☑	6.724e-2		N/A		290.100
856	3	Unknown	BEG-2	Qualifiers	BEG	N/A	2.53	7.975e5	2.62	0.09	☑	6.157e-2		N/A		290.100
863	3	Unknown	COCAETILENE-1	Quantifiers	COCAETILENE	N/A	3.09	7.536e6	3.16	0.07	☑	8.110e-2		N/A		318.300
864	3	Unknown	COCAETILENE-2	Qualifiers	COCAETILENE	N/A	3.09	1.036e6	3.16	0.07	☑	7.678e-2		N/A		318.300
865	3	Unknown	COCAINA-1	Quantifiers	COCAINA	N/A	2.86	3.544e7	2.94	0.08	☑	4.852e-1		N/A		304.200
866	3	Unknown	COCAINA-2	Qualifiers	COCAINA	N/A	2.94	4.595e6	2.94	0.00	☑	4.283e-1		N/A		304.200

Figure 9: Optimized DOA screening method leads to positive identification of drugs of abuse in two real hair case samples. SCIEX OS Software results table showing positive identification of a number of drugs and drug metabolites in case sample 3 (top panel) along with XICs of the positively detected analytes (bottom panel). THC, cocaine and two of its main metabolites; benzoylecgonine and cocaethylene were positively identified in case sample number 3 at concentration of 0.2920, 0.4568, 0.06450 and 0.07894 ng/mg, respectively.

Ethyl Glucuronide (EtG) results

Lastly, the same sample preparation procedure were used to extract EtG, one of the endogenous ethanol metabolites and a biomarker of alcohol intake. Prior to extraction, the control hair samples were spiked with EtG and EtG-D9 (IS), resulting in final concentrations ranging from 20 to 300 pg/mg for EtG and 200 pg/mg for EtG-D5, respectively. The current EtG calibration range covers the cut-off value of 30 pg/mg which corresponds to the concentration of EtG in hair samples used to discriminate between social and heavy drinkers (as measured in the 0-3 cm proximal hair segment).[1] A data processing method was developed in SCIEX OS Software to extract the peaks corresponding to EtG and build a data processing method.

Figure 10 shows the extracted ion chromatograms (A) and the calibration curves (B) resulting from the calibration series performed for EtG. Four calibration levels ranging from 20 to 300 pg/mg were used to generate the calibration curves. The regression curves showcase excellent correlation of the generated regression curves with R^2 values of 0.99902 and 0.99917 for each of the EtG transitions.

To further assess the robustness of the developed method for EtG detection, real hair samples were analyzed. Those case samples originated from subjects with known recent alcohol consumption as well as subjects restraint from alcohol consumption for at least 60 days prior to hair collection. Figure 11 summarizes the results of three case samples that tested positive for EtG. It was detected in these case samples at concentration of 7.6070 (case sample number 1) , 10.660 (case sample number 2) and 1.6071 pg/mg (case sample number 3).

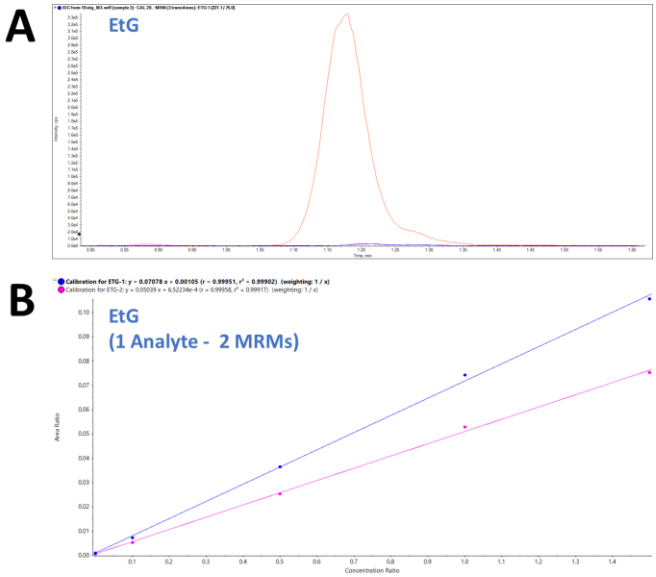
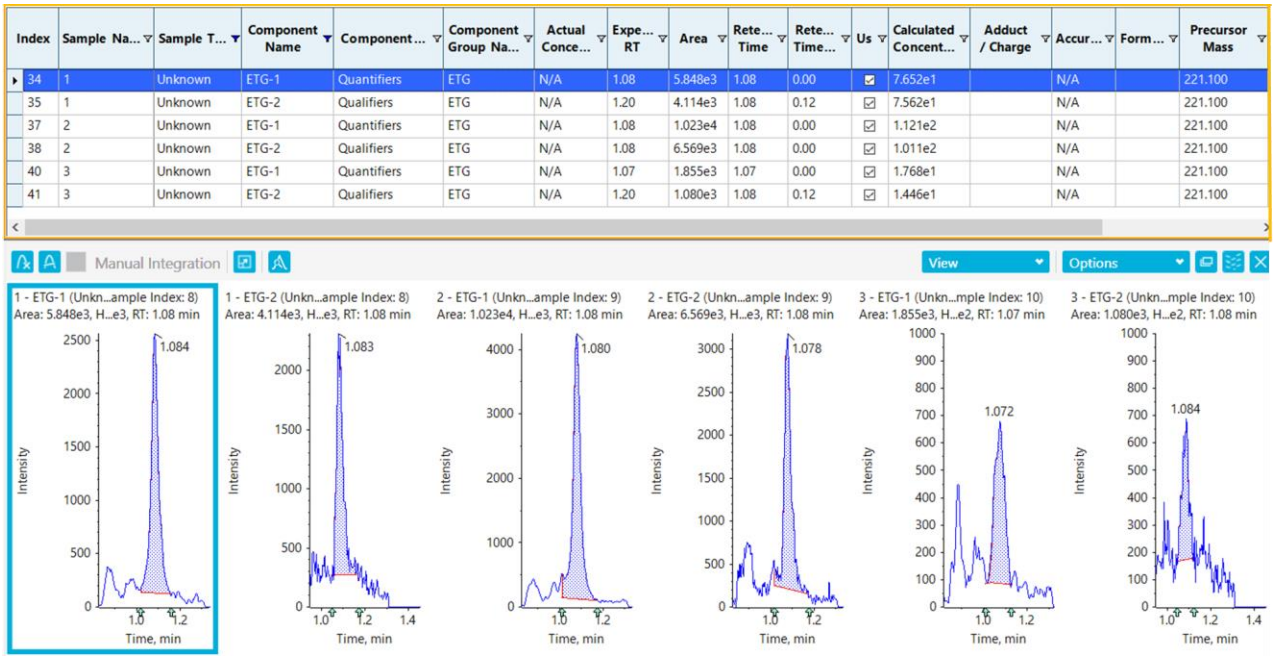


Figure 10. Robust detection of EtG, a direct alcohol metabolite used as an indicator of alcohol consumption. (A) XICs showing each MRM transition monitored for EtG. (B) Calibration curves resulting from the calibration series for EtG showing excellent linear response (R^2 values > 0.99) across the calibration range from 20 to 300 pg/mg.



Index	Sample Na...	Sample T...	Component Name	Component ...	Component Group Na...	Actual Conce...	Expe... RT	Area	Rete... Time	Rete... Time...	Us	Calculated Concent...	Adduct / Charge	Accur...	Form...	Precursor Mass
34	1	Unknown	ETG-1	Quantifiers	ETG	N/A	1.08	5.848e3	1.08	0.00	☑	7.652e1		N/A		221.100
35	1	Unknown	ETG-2	Qualifiers	ETG	N/A	1.20	4.114e3	1.08	0.12	☑	7.562e1		N/A		221.100
37	2	Unknown	ETG-1	Quantifiers	ETG	N/A	1.08	1.023e4	1.08	0.00	☑	1.121e2		N/A		221.100
38	2	Unknown	ETG-2	Qualifiers	ETG	N/A	1.08	6.569e3	1.08	0.00	☑	1.011e2		N/A		221.100
40	3	Unknown	ETG-1	Quantifiers	ETG	N/A	1.07	1.855e3	1.07	0.00	☑	1.768e1		N/A		221.100
41	3	Unknown	ETG-2	Qualifiers	ETG	N/A	1.20	1.080e3	1.08	0.12	☑	1.446e1		N/A		221.100

Figure 11: EtG positive real hair case samples demonstrating the overall robustness of the method for EtG detection. SCIEX OS Software results table showing sample information for the three real hair case samples in which EtG was detected (top) along with XICs corresponding to the detection of each MRM transition monitored for EtG. The table include the peak area as well as the calculated concentration for each MRM transition monitored. SCIEX OS Software enabled the ability to select the positive real hair case samples using the filtering option and include the peak are as well as the calculated concentration for each MRM transition monitored. Visualization of the XICs to review integration of the peaks was easily accomplished at the click of a button.

These results demonstrate that the current method is able to differentiate abstinent subjects from those with recent alcohol consumption (social drinkers rather than heavy drinkers in the presented case samples). Overall, the presented workflow provides an accurate and reliable quantitative measure of EtG concentration from real hair samples. In addition, this method provides the ability to monitor alcohol consumption of up to 60 days prior to hair collection.

Conclusions

A comprehensive workflow combining the sensitivity of the SCIEX QTRAP 6500+ LC-MS/MS System with a simple extraction procedure enabled detection of a wide variety of drugs and drug metabolites in hair samples. The developed workflow was shown to enable accurate identification and sensitive quantification of a wide range of chemically-diverse analytes included in three different panels. The broad applicability of the sample preparation procedure allows accurate quantification of these analytes in real head hair samples at high pg/mg to low ng/mg levels, proving the robustness of the extraction method and the rugedness of the the developed workflow.

- 14-step sample preparation procedure enabled extraction of of a wide array of chemically-diverse drugs and drug metabolites from real hair samples
- Optimized LC methods enabled separation of structurally-related and isobaric compounds present in each of the three panels with a high level of selectivity
- The three separate workflows showed excellent linearity across their respective calibration range, resulting in R^2 values >0.98 for all the analytes included in the panels
- Developed workflows enabled high pg/mg to low ng/mg detection limits of drugs and drug metabolites while maintaining linearity across the calibration range
- Extraction method was applied to real hair case samples from subjects who had reported recent drug and alcohol consumption
- Developed methods enabled identification and quantification of high pg/mg detection limits of NPS, DOA and EtG in these real hair samples

Table 1. List of analytes organized by drug class included in the Novel Psychoactive Substance (NPS) panel.

Analytes	Analytes	Analytes
Synthetic Cannabinoids	***Phenethylamines***	***Synthetic Opioid Metabolites***
5-fluoride ADB	*3.4 -MDPV*	*cis-3-methil Norfentanyl*
5-chloro-AB-PINACA	*5-EAPB*	*4-ANPP*
5-chloro-THJ 018	*5-MAPB*	*Acetylnorfentanylloxalate*
5-fluoro-AKB48	*6-APB*	*Butyryrylfentanylcarboxy metabolite*
5-fluoro-APP-PICA	*6-MAPB*	*Butyryl-nor-fentanyl*
5-fluoro-APP-PINACA	*Butylone*	*Despropionyl para-Fluorofentanyl*
5-fluoro-CUMYL-PINACA	*Metylone*	*Furanyl-nor-fentanyl*
5-fluoro-NNEI 2-naphtyl isomer		*Methoxyacetylnorfentanyl*
AB-CHEMISTRY	***Synthetic Opioids***	*Norfentanyloxalate*
AB-FUBINACA	*Fentanyl*	*Valerylfentanylcarboxy metabolite*
ADB-FUBINACA	*Trans-3-methyl Norfentanyl*	
APP-FUBINACA	*Acetylfentanyl*	***Tryptamines***
CUMYL-PeGACLONE	*Alfentanyl*	*4-acetoxy DiPT*
MDMB-CHEMISTRY	*Butyrylfentanyl*	*4-acetoxy DMT*
MMB2201	*Carfentanyl*	*4-hydroxy DET*
JWH-122	*Cyclopropyl-Fentanyl*	*5-methoxy AMT*
JWH 251	*Fentanyl, New1*	*5-methoxy DALT*
JWH -016	*Phenyl-Fentanyl*	*5-methoxy DMT*
JWH -007	*Phenyl-acetyl-Fentanyl*	*5-methoxy DPT*
JWH -019	*Hydroxyl-Fentanyl*	*5-methoxy MiPT*
JWH -3022	*Hydroxyl-thio-Fentanyl*	*N,N-DMT*
JWH -081	*Phenyl-Fentanyl*	*Psilocybin*
JWH -398	*Trans-3-methil Norfentanyl*	*Alpha-Ethyltryptamine*
JWH -147		
JWH -098	***Methylphenidate***	***Amino Acid (Serotonin Precursor)***
JWH -203	*Ritalic acid*	*5-hydroxytryptophan*
JWH -307		
JWH-210	***Cathinone***	
AM-694	*Methedrone*	
AM-2201		
RCS 4	***Piperidine***	
RCS 8	*Ethylphenidate*	

Table 2. List of analytes organized by drug class included in the Drugs of Abuse (DOA) panel.

Analytes	Analytes
Stimulants	***Opioids***
Cocaine	*Morphine*
Cocaethylene	*Monoacetylmorphine*
Ecgonine Methyl Ester (EME)	*Codeine*
Amphetamine	*Tramadol*
Methamphetamine	*Oxycodone*
MDMA	*Fentanyl*
MDEA	
Ketamine	***Cannabinoids***
Norketamine	*THC*
	CBD
Rx Depressants	*CBN*
Methadone	
Buprenorphine	***Benzodiazepine***
Zolpidem	*Clobazam*
EDDP	

References

1. P. Kintz, Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2011, *Forensic Sci. Int.* **218,** 2.

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