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Simultaneous determination in hair of multiclass drugs of abuse (including THC) by ultrahigh performance liquid chromatography - tandem mass spectrometry

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(Article begins on next page)



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Abstract

A simple procedure for the quantitative determination in hair samples of 13 common drugs of abuse or metabolites (morphine, 6-acetylmorphine, codeine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, benzoylecgonine, cocaine, buprenorphine, methadone and Δ^9 -tetrahydrocannabinol) has been developed and fully validated. The analytes were extracted from the matrix by a simple overnight incubation with methanol at 55 °C. An aliquot of the extract was directly injected into an ultra-high performance liquid chromatography system equipped with Waters Acquity UHPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μ m). The mobile phase eluted with a linear gradient (water/formic acid 5 mM:acetonitrile; v:v) from 98:2 to 0:100 in 4.5 min, followed by isocratic elution at 100% B for 1.0 min. The flow rate was 0.6 mL/min and the total run time was 8.0 min including re-equilibration at the initial conditions. The compounds were revealed by a triple quadrupole mass spectrometer operating in the selected reaction monitoring mode. The absence of matrix interferents, together with excellent repeatability of both retention times and relative abundances of diagnostic transitions, allowed the correct identification of all analytes tested. The method proved linear in the interval from the limit of quantification to 5.0 ng/mg (1.0 ng/mg for Δ^9 -tetrahydrocannabinol) with correlation coefficient values ranging from 0.9970 to 0.9997. Quantitation limits were below the cut-off values recommended by the Society of Hair Testing and ranged from 0.02 to 0.08 ng/mg. Application of the present UHPLC–MS/MS procedure and instrumentation to hair analysis allows high sample-throughput, together with excellent sensitivity and selectivity, in workplace drug-screening controls and forensic investigations. These qualities, combined with minimal sample workup, make the cost of this screening affordable for most private and public administrations.

Highlights

► Hair analysis is a tool to evaluate drug exposure in several application fields. ► We present a simple UHPLC–MS/MS method to detect 13 common drugs of abuse in hair. ► The method proved excellent analytical performances and drastic reduction of time. ► High sample throughput is essential for laboratories. ► The increased global productivity makes the workplace testing of hair affordable.

Keywords

- Hair;
- Ultra-high performance liquid chromatography;
- Multidrugs;
- Validation

1. Introduction

The determination at low concentration of common psychotropic drugs is increasingly requested in hair samples for the retrospective investigation of habitual drug abuse and dependence as well as in other toxicological investigations [1]. Nowadays, hair analysis is a widely used tool to evaluate drug exposure in several application fields, such as workplace drug testing, driving re-licensing, drug abuse history and withdrawal control, drug-facilitated crimes, post-mortem toxicology, pre-natal exposure to drugs, doping control, therapeutic drug monitoring of pharmaceuticals [2], [3], [4], [5], [6], [7], [8], [9] and [10]. To meet the high demand for drug screening in hair samples, toxicology laboratories are forced to update their procedures, in order to target an increasing number of drugs but also achieve rapid, simple and sensitive analyses with reduced work for sample preparation and instrumental processing, so as to increase overall sample-throughput. Different methods have been proposed for testing class-specific groups of compounds, but only a few papers describe extensive screening of multiclass drugs [11], [12] and [13], among which Δ^9 -tetrahydrocannabinol (THC) was never included. The various analytical methods employed to test hair extracts for abused drugs have been recently reviewed [14]. These methods include derivatization followed by gas chromatography/chemical ionization mass spectrometry [15], liquid chromatography with various mass spectrometers, such as triple quadrupoles [16], ion traps [17], time-of-flight [18] and hybrid linear ion trap-orbitrap mass spectrometers [19]. The use of direct mass spectrometric techniques such as ambient ionization mass spectrometry [20] and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) [21] have also been described for hair drug testing.

Most of the cited methods were implemented for the determination of a few analytes, as is evident from the synoptic table reported in the cited review [14]. In order to perform wide range screening of abused drugs, more than one procedures are likely to be utilized, with direct impact on efficiency and costs. In particular, extensive application of hair analysis in workplace drug testing is often prevented by its cost, crucially high for public administrations.

Our goal was to develop and validate a sensitive multi-class and multiresidual screening method for drugs of abuse or metabolites in hair samples using a dedicated UHPLC–MS/MS protocol. In comparison with the previously reported procedures, the present method used a simple sample extraction and direct injection into the UHPLC–MS system, avoiding both solid-phase and liquid–liquid extraction. Furthermore, the utilization of recent UHPLC–MS/MS technology allowed a drastic reduction of the analysis time, without resolution loss. The method proved simple, accurate, rapid and highly sensitive, allowing the simultaneous detection of all the most common drugs, including THC, and high sample throughput, resulting in significantly reduced costs of analysis.

2. Experimental

2.1. Chemicals and reagents

Morphine, 6-monoacetylmorphine (6-MAM), codeine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), N-methyl-3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), benzoylecgonine (BZE), cocaine, buprenorphine, methadone, Δ^9 -tetrahydrocannabinol (THC), cocaine-*d3* (COC-*d3*), amphetamine-*d6* (AMP-*d6*), morphine-*d3* (MOR-*d3*), benzoylecgonine-*d3* (BZE-*d3*), 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol-*d3* (THCmet-*d3*) were purchased from LGC Promochem (Milan, Italy). Dichloromethane, methanol, formic acid, acetonitrile were provided by Sigma–Aldrich (Milan, Italy). Ultra-pure water was obtained using a Milli-Q® UF-Plus apparatus (Millipore, Bedford, MA, USA). Stock standard solutions were stored at $-20\text{ }^{\circ}\text{C}$ until used. Working standard solutions were prepared at $10\text{ }\mu\text{g/mL}$ final concentration by dilution with methanol.

2.2. Sample preparation

About 50 mg of hair was twice-washed with dichloromethane (2 mL, vortex mixed for 3 min). After complete removal of solvent wash, the hair was dried at room temperature by a gentle nitrogen flow and subsequently cut with scissors into 1–2 mm segments. Hair samples were fortified with 2 μL of an internal standards mixture yielding a final concentration of 0.6 ng/mg. After the addition of 2 mL of methanol, the samples were incubated at $55\text{ }^{\circ}\text{C}$ for 15 h without stirring. Lastly, the organic phase was collected and an aliquot of 1 μL was directly injected into the UHPLC–MS/MS system.

2.3. Instrumentation

Analyses were performed using a Shimadzu LC-30A Series system (Shimadzu, Duisburg, Germany), interfaced to an API 5500 triple quadrupole mass spectrometer (Applied Biosystem/Sciex, Darmstadt, Germany) equipped with an electrospray Turbo Ion source operating in positive-ion mode. A Waters Acquity UHPLC BEH C18 column (100 mm \times 2.1 mm, 1.7 μm), protected by a C18 guard column, was used for the target analytes separation. The column oven was maintained at $50\text{ }^{\circ}\text{C}$ and the elution solvents used were water/formic acid 5 mM (solvent A) and acetonitrile (solvent B). The mobile phase eluted under the following linear gradient conditions (A:B; v:v): from 98:2 to 0:100 in 4.5 min, followed by isocratic elution at 100% B for 1.0 min. The flow rate was 0.6 mL/min and the total run time was 8.0 min including re-equilibration at the initial conditions. The mass analyzers were operated in the selected reaction monitoring (SRM) mode. In order to establish appropriate SRM conditions, optimization of the mass spectrometer was conducted by direct infusion of the analytes into the electrospray ionization capillary and the declustering potential (DP) was adjusted to maximize the intensity of the protonated molecular

species. The collision offset (CE) voltage values were selected so as to preserve approximately 10% of each precursor ion. Nitrogen was employed as the collision gas (5×10^{-3} Pa). The ESI source was held at 550 °C. Precursor ions and the corresponding product ion SRM transitions employed for all analytes and internal standards are presented in Table S1, supplementary data.

2.4. Method validation

The method was validated for linearity, selectivity, quantitation limits (LOD and LOQ), intra/inter-day precision, and accuracy [22] and [23]. Carry-over and matrix effect phenomena were also evaluated.

2.4.1. Selectivity

A pool of five different blank hair samples obtained from different healthy volunteers (two females, three males) was prepared and analyzed as described above. The occurrence of possible interferences from endogenous substances was tested by monitoring the SRM chromatograms characteristic for each investigated compound at the expected retention time interval.

2.4.2. Identification criteria and repeatability of diagnostic fragment ions relative abundances

Identification criteria for the analytes were established according to CE/2002/657 decision and 2006 SOFT/AAFS guidelines criteria [22]. The repeatability of relative peak intensities for the transitions of each analyte was determined on five spiked hair samples at three concentration levels (0.2, 1.0 and 5.0 ng/mg; 0.04, 0.2 and 1.0 ng/mg for THC). Retention time precision at each concentration was also determined.

2.4.3. Linearity, limit of detection and limit of quantitation

The linear calibration model was checked by analyzing (three replicates) blank hair samples spiked with standard solutions at six concentration levels for each analyte (Table 1). Linearity was evaluated using the least squares regression method. Quantitative data resulting from area counts were corrected using the IS signal areas. The limit of detection (LOD) was estimated as the analyte concentration whose response provided a S/N value equal to 3, as determined from the least abundant among the qualifier ions; LOD was extrapolated from S/N values of the three lowest concentrations of the calibration curve. The calculated LODs were then experimentally confirmed by analyzing spiked samples at LODs concentration for all analytes. The limit of quantification (LOQ) was estimated based on the S/N ratio to be equal or greater than 10 [23].

Table 1. Calibration levels, R^2 values for calibration curve and matrix effect; LODs and LOQs values of the 13 analytes investigated.

Compound	Internal standard	Calibration levels (ng/mg)	Regression coefficient (R^2)	Matrix effect ^a		LOD (ng/mg)	LOQ ^b (ng/mg)
				Mean ($\pm\%$)	CV%		
Morphine	MOR- <i>d3</i>	0.20–5.0	0.9985	-6.7	4.7	0.009	0.03
Codeine	MOR- <i>d3</i>	0.20–5.0	0.9991	-14.1	7.0	0.012	0.04
Amphetamine	AMP- <i>d6</i>	0.20–5.0	0.9980	+0.2	5.6	0.027	0.08
6-MAM	MOR- <i>d3</i>	0.20–5.0	0.9985	-3.9	4.9	0.007	0.02
MDA	AMP- <i>d6</i>	0.20–5.0	0.9990	+0.7	6.5	0.021	0.06
Methamphetamine	AMP- <i>d6</i>	0.20–5.0	0.9997	-2.8	6.4	0.011	0.03
MDMA	AMP- <i>d6</i>	0.20–5.0	0.9987	+0.6	9.3	0.006	0.02
MDEA	AMP- <i>d6</i>	0.20–5.0	0.9970	+1.6	3.0	0.012	0.04
Benzoylcegonine	BZE- <i>d3</i>	0.20–5.0	0.9994	-4.3	4.1	0.007	0.02
Cocaine	COC- <i>d3</i>	0.20–5.0	0.9987	-4.7	12.8	0.011	0.03
Buprenorphine	MOR- <i>d3</i>	0.20–5.0	0.9986	-2.7	11.6	0.027	0.08
Methadone	BZE- <i>d3</i>	0.20–5.0	0.9982	+1.0	5.9	0.011	0.03
THC	THCmet- <i>d3</i>	0.04–1.0	0.9981	-21.6	24.9	0.012	0.04

^aMatrix effect was evaluated using five different sources of hair.

^bCalculated LOQ.

2.4.4. Matrix effect

The matrix effect was calculated as the mean value obtained from five different hair sources. Hair samples were spiked after the extraction step at the final concentration of 1.0 ng/mg (0.2 ng/mg for Δ^9 -THC). For each analyte, the chromatographic peak area was compared with the peak area of standard solutions prepared in methanol. For each sample, analyses were repeated three times. Variability of matrix effect among different hair samples was expressed as CV%.

2.4.5. Precision and accuracy

For all analytes, intra-day precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %) were evaluated at LOQ and at different concentration levels. Ten replicates of blank hair samples spiked with the standard solutions at the final concentration of LOQs, 0.2, 1.0 and 5.0 ng/mg (0.04, 0.2 and 1.0 ng/mg for Δ^9 -THC) and analyzed by the described method. Inter-day precision and accuracy were evaluated by preparing and analyzing for three

consecutive days one set of ten hair samples spiked with target compounds at LOQ and at the final concentration of 1.0 ng/mg (0.2 ng/mg for Δ^9 -THC). Standard criteria designated satisfactory assay precision when CV% values were below 25% for lower concentrations and below 15% for upper concentrations. Satisfactory accuracy was achieved when the experimentally determined concentrations lied within $\pm 25\%$ from the expected values. Bias % ($\%B$) was estimated as the percent difference between the average value of a set of measurements (X) and the “true value” (T) following the formula $\%B = (100/T) \times (X - T)$. The parameters most commonly changing in everyday toxicological analysis, namely sample volume, reagent batch and operator, were deliberately varied to test if satisfactory accuracy was maintained.

2.4.6. Carry-over

The background chromatographic profiles for each analyte were monitored during the analysis of blank hair sample injected for five times after the chromatographic run of a spiked blank hair sample containing all the analytes at 5.0 ng/mg concentration. To assure the absence of carry-over, the signal to noise ratio (S/N) for each transition had to be lower than 3.

3. Results and discussion

3.1. Method validation

3.1.1. Selectivity

The SRM chromatograms obtained from five blank hair samples presented no peaks arising from endogenous interferences (i.e. S/N < 3) at the expected retention time for all analytes. This demonstrated that the method is selective for the tested compounds and free from positive interference from hair components and column bleeding.

3.1.2. Identification criteria

The SRM transitions selected for each analyte provided at least 4 identification points, while the substantial stability of their relative abundances proved compliant to the unambiguous identification of all analytes included in the assay, in agreement with CE/2002/657 decision and 2006 SOFT/AAFS guidelines criteria[22]. Moreover, the intra-day precision values for retention times measured at 0.2, 1.0 and 5.0 ng/mg concentration (0.04, 0.2 and 1.0 ng/mg for THC) were below 0.5%, confirming that retention times are repeatable and not affected by the analytes concentration.

3.1.3. Linearity, limit of detection and limit of quantitation

The SRM protocol described (see Table S1) was used to build the calibration plots for all thirteen analytes. Adequate linearity was observed for all compounds. Table 1 reports the resulting R^2 values, ranging from 0.9970 and 0.9997 and indicating good fit and linearity of the calibration curves. Table 1 also reports LOD and LOQ values, calculated from the analysis of multiple blank samples. Detection of analytes at LOD levels was confirmed experimentally (see Fig. 1). LOD values ranged between 0.006 ng/mg and 0.027 ng/mg while LOQ values were estimated between 0.02 ng/mg and 0.08 ng/mg. When compared to existing procedures using similar techniques [14], the present method provided equal or lower LODs and LOQs. This demonstrates that the inclusion of a large set of analytes within the screening protocol and the simplified sample treatment did not affect significantly the method sensitivity, while the newest UHPLC technology coupled with the last generation of mass spectrometers guarantees short analysis time with concurrent improvement of analytical performances.

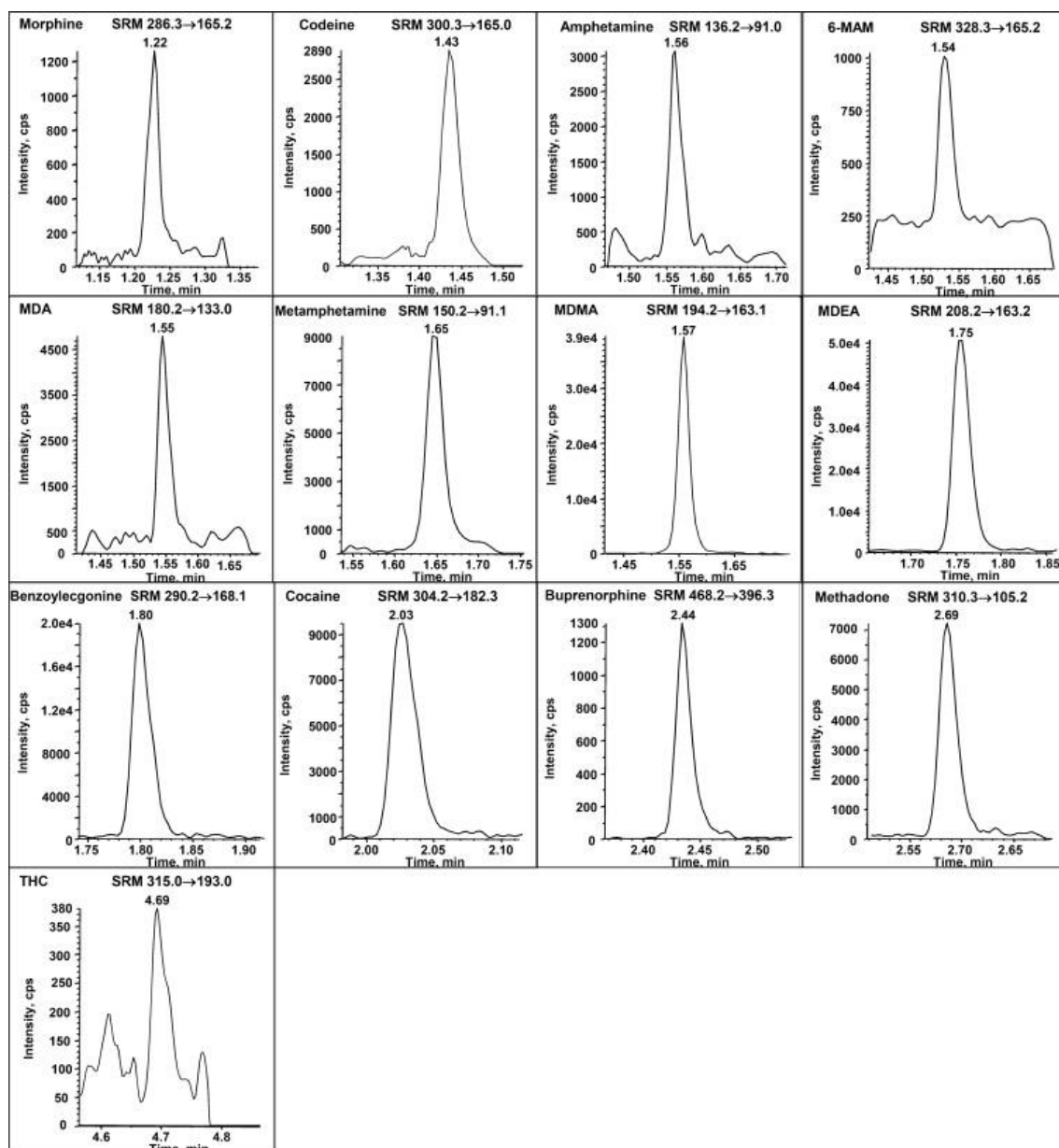


Fig. 1. MRM chromatograms of a blank hair sample spiked at LOD.

3.1.4. Matrix effect

The variability among different hair samples was acceptable ($CV\% < 25\%$, as shown in [Table 1](#)), in consequence we pooled together the five sources of hair to perform validation experiments such as precision and accuracy. The effect of real hair matrix did not appear significant for most of the analytes tested (see [Table 1](#)). A moderate ion suppression was observed for morphine, 6-MAM, benzoylcegonine and cocaine, while the matrix influence was minor for methadone, all the amphetamines and buprenorphine. Only codeine and Δ^9 -THC underwent a considerable ion suppression from keratinic matrix (values $\leq -10\%$). To compensate as much as possible the matrix

effects present in real hair samples analysis, all calibration and validation tests were conducted on a pool of human hair samples, spiked with standard analytes solutions. The good linearity observed in the calibration plots demonstrated however that the observed matrix effect is proportionally constant, i.e. does not depend on the analytes' concentrations.

3.1.5. Precision and accuracy

Intra- and inter-day data on precision and accuracy are reported in Table 2. The results show satisfactory intra-day repeatability, as the percent variation coefficient (CV%) is lower than 15% for all the spiked analytes at LOQ, low, medium and high concentration. In particular, intra-day precision exhibits CV% values ranging between 0.7% and 14.9%.

Table 2. Intra/inter-day precision (CV%) and accuracy (bias %) for each analyte tested.

Compound	Intraday (<i>n</i> = 30)								Interday (<i>n</i> = 30)			
	Precision (CV%)				Accuracy (bias%)				Precision (CV%)		Accuracy (CV%)	
	LOQ	Low ^a	Medium ^b	High ^c	LOQ	Low ^a	Medium ^b	High ^c	LOQ	Medium ^b	LOQ	Medium ^b
Morphine	8.1	3.9	4.4	3.5	-18.5	+6.0	-5.4	-13.3	8.8	9.9	-24.3	+8.4
Codeine	6.4	3.2	2.6	3.9	-8.0	+10.0	+3.0	-13.2	7.4	8.1	-22.0	-8.4
Amphetamine	5.8	6.3	5.2	9.7	+10.0	+4.0	-2.2	-12.8	5.4	12.0	+5.6	+18.4
6-MAM	14.9	5.0	6.2	2.4	-14.0	+9.0	+7.0	-10.8	17.4	11.0	-14.8	+16.4
MDA	8.1	7.1	5.3	5.9	+3.5	-1.0	-1.0	-6.7	9.1	11.6	-3.0	+4.8
Methamphetamine	13.0	2.6	4.7	5.6	+47.5	+7.0	+10.2	-12.5	21.5	15.5	+52.8	+12.8
MDMA	2.7	5.7	0.7	5.6	+22.0	-4.0	+14.8	-13.8	25.3	16.9	+31.3	+6.0
MDEA	2.6	10.9	8.8	4.4	+14.0	-1.0	+18.0	-11.0	5.5	22.5	+16.0	+14.8
Benzoylcegonine	1.4	3.9	9.0	4.6	-18.0	+6.0	+8.0	+0.5	4.9	17.8	-22.7	+18.0
Cocaine	3.0	9.9	4.7	4.5	+15.0	+9.0	+14.8	-6.5	5.1	12.0	+10.7	-6.4
Buprenorphine	6.7	2.7	2.7	6.9	-3.0	+3.0	-4.2	-9.8	4.3	22.4	-2.2	+9.2
Methadone	7.7	7.5	4.8	5.9	+18.5	+9.0	+9.4	-2.2	10.3	9.4	+26.3	+19.6
THC	12.4	12.4	4.1	5.9	+10.0	+10.0	+1.0	-3.0	20.0	10.8	+14.0	+6.0

^aLow concentration: 0.2 ng/mg (0.04 ng/mg for THC).

^bMedium concentration: 1.0 ng/mg (0.2 ng/mg for THC).

^cHigh concentration: 5.0 ng/mg (1.0 ng/mg for THC).

The intra-day accuracy results proved better than acceptable in most cases, particularly at the lowest concentration tested, where percent bias values ranged from -4.0% (MDMA) to +10.0% (codeine and THC). This upshot is particularly significant, as the 0.2 ng/mg level correspond to the generally accepted cut-off for many analytes, such as opiates, methadone and amphetamines. Indeed, high accuracy at cut-off levels is an essential pre-requisite in a screening method. At intermediate spiking concentrations the bias ranged from -5.4% (morphine) to +18.0% (MDEA), whereas at the highest concentration level the upper and lower limits were +0.5% (benzoylecgonine) and -13.8% (MDMA). On the whole, all the experimental bias values were largely below the acceptable limit of $\pm 25\%$ at all concentrations. Only for methamphetamine, the intra-day accuracy calculated at LOQ showed a significant overestimation (+47.5%).

At the intermediate concentration level (1.0 ng/mg for all analytes, 0.2 ng/mg for Δ^9 -THC), also the inter-day precision proved satisfactory, as the CV% values ranged from 8.1% for codeine to 22.5% for MDEA, likewise the intra-day accuracy, ranging from -8.4% to +19.6. As for intra-day results, also inter-day precision data calculated at LOQ were satisfactory for all compounds while methamphetamine, MDMA and methadone were largely overestimated. This does not represent a limit to the applicability of the method, since a screening method requires a more accurate quantification at cut-off levels rather than at LOQs.

3.1.6. Carry-over

The background chromatographic profiles of the main transitions for each analyte, monitored during the analysis of blank urine injected after highly spiked samples, did not show the presence of any significant signal (i.e. the S/N value was always <3) at the retention times expected for the tested analytes. Therefore, the occurrence of carry-over effects was excluded.

3.1.7. Application to real cases

Our laboratory is continuously using the present screening method for the routine analysis of real samples, mainly from driving re-licensing, drug abuse history and withdrawal control, post-mortem toxicology. When one or more of the molecules included in this screening are identified, a confirmation analysis is usually performed. In 2011, this fast and comprehensive screening method allowed us to manage a load of 3832 samples (head, pubic, axillary or chest hair). We also successfully participated to external Proficiency Tests including those organized by the Society of Hair Testing, GTFCh and Istituto Superiore di Sanità (Rome, Italy).

The two following examples of multiple positive identifications are reported, in order to demonstrate the practical usefulness and general applicability of this method. In [Fig. 2](#) a chromatogram of a sample positive to MDMA, cocaine, BZE and methadone is reported. In [Fig. 3](#) a chromatogram of a sample positive to morphine, 6-MAM, codeine and THC is reported.

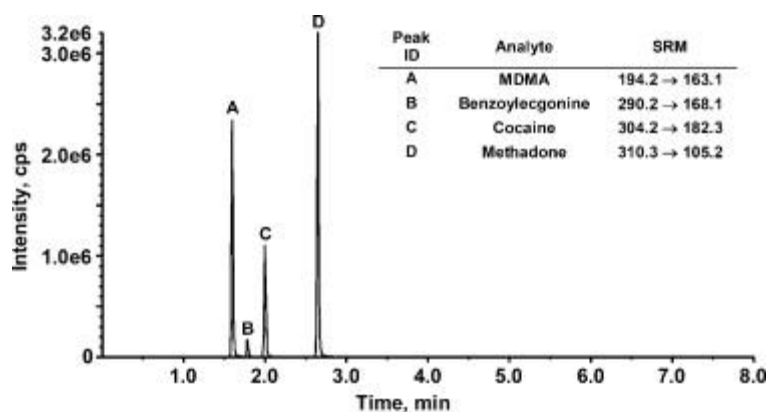


Fig. 2. MRM chromatogram of a real sample positive to MDMA, cocaine, BZE and methadone.

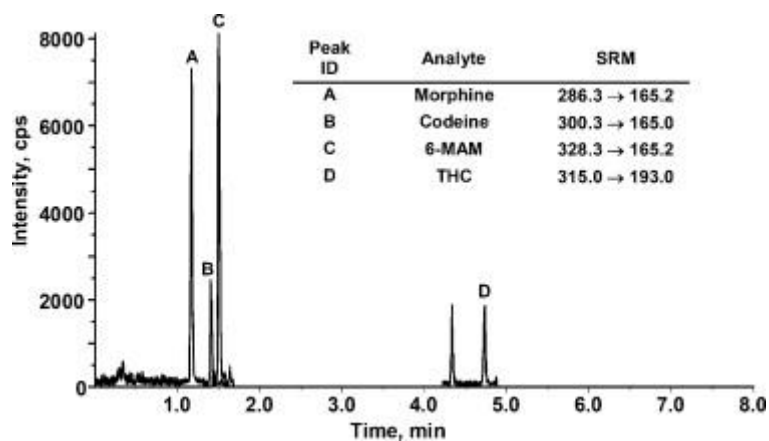


Fig. 3. MRM chromatogram of a real sample positive to morphine, 6-MAM, codeine and THC.

4. Conclusions

A simple and fully validated procedure is described for the simultaneous screening and determination in hair samples of morphine, 6-acetylmorphine, codeine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, benzoylcegonine, cocaine, buprenorphine, methadone and Δ^9 -tetrahydrocannabinol. The main features of the proposed method are the speed of sample processing, the wide range of drugs considered and the comprehensive analytical sensitivity toward them. The ease of sample treatment, together with the simultaneous determination of multiclass substances, including THC, in a single run of 5.5 min plus 2.5 min of column re-equilibration time, make the procedure highly effective for large sample loadings. The modern SRM technique and instrumentation provided improved sensitivity, allowing us to avoid the sample pre-concentration step and resulting in a drastic reduction of sample handling. On the other hand, the

utilization of UHPLC–MS/MS technology drastically reduced the time required for instrumental analysis, without sacrificing the chromatographic resolution, nor the accuracy and precision for quantitative determinations.

In general, high sample throughput achievable by the present method considerably reduces the overall analysis cost, including investment pay-back, making it affordable, especially for public administrations, in workplace testing. Moreover, the analytical performances are high and relatively uniform for all the studied analytes, so that the present protocol may find easy application in routine analysis for toxicological investigations.

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References

- [1] P. Kintz **Analytical and Practical Aspects of Drug Testing in Hair** CRC Press, Boca Raton, FL, USA (2007)
- [2] F. Pragst, M.A. Balikova *Clin. Chim. Acta*, 370 (2006), p. 17
- [3] E.J. Cone *Forensic Sci. Int.*, 121 (2001), p. 7
- [4] Y.H. Caplan, B.A. Goldberger *J. Anal. Toxicol.*, 25 (2001), p. 396
- [5] P. Kintz, M. Villain, V. Cirimele *Ther. Drug Monit.*, 28 (2006), p. 442
- [6] P. Kintz *Anal. Bioanal. Chem.*, 388 (2007), p. 1467
- [7] J. Lozano, O. García-Algar, O. Vall, R. de la Torre, G. Scaravelli, S. Pichini *Ther. Drug Monit.*, 29 (2007), p. 711
- [8] M. Barroso, M. Dias, D.N. Vieira, M. López-Rivadulla, J.A. Queiroz *Anal. Biochem. Chem.*, 396 (2010), p. 3059
- [9] E. Gerace, A. Salomone, S. Pellegrino, M. Vincenti *Forensic Sci. Int.*, 215 (2012), p. 121
- [10] A. Salomone, E. Gerace, D. Di Corcia, G. Martra, M. Petrarulo, M. Vincenti *Int. J. Legal Med.*, 126 (2012), p. 451
- [11] R. Kronstrand, I. Nystrom, J. Strandberg, H. Druid *Forensic Sci. Int.*, 145 (2004), p. 183
- [12] A. Pelander, J. Ristimaa, I. Rasanen, E. Vuori, I. Ojanpera *Ther. Drug Monit.*, 30 (2008), p. 717
- [13] M.K. Klose Nielsen, S.S. Johansen, P.W. Dalsgaard, K. Linnet *Forensic Sci. Int.*, 196 (2010), p. 85

- [\[14\]](#) M. Wada, R. Ikeda, N. Kuroda, K. Nakashima *Anal. Bioanal. Chem.*, 397 (2010), p. 1039
- [\[15\]](#) Y.H. Wu, K.L. Lin, S.C. Chen, Y.Z. Chang *J. Chromatogr. B*, 870 (2008), p. 192
- [\[16\]](#) O. Quintela, E. Lendoiro, A. Cruz, A. de Castro, A. Quevedo, C. Jurado, M. López-Rivadulla *Anal. Bioanal. Chem.*, 396 (2010), p. 1703
- [\[17\]](#) F. Bucelli, A. Fratini, P. Bavazzano, N. Comodo *J. Chromatogr. B*, 877 (2009), p. 3931
- [\[18\]](#) J.C. Dominguez-Romero, J.F. García-Reyes, A. Molina-Díaz *J. Chromatogr. B*, 879 (2011), p. 2034
- [\[19\]](#) S. Vogliardi, D. Favretto, M. Tucci, G. Stocchero, S.D. Ferrara *Anal. Bioanal. Chem.*, 400 (2011), p. 51
- [\[20\]](#) A.U. Jackson, J.F. Garcia-Reyes, J.D. Harper, J.S. Wiley, A. Molina-Díaz, Z. Ouyang, .G. Cooks *Analyst*, 135 (2010), p. 927
- [\[21\]](#) S. Vogliardi, D. Favretto, G. Frison, S. Maietti, G. Viel, R. Seraglia, P. Traldi, S.D. Ferrara *Anal. Bioanal. Chem.*, 396 (2010), p. 2435
- [\[22\]](#) SOFT, AAFS, Forensic Toxicology Laboratory Guidelines, SOFT and AAFS (01.11.06).
- [\[23\]](#) F.T. Peters, O.H. Drummer, F. Musshoff *Forensic Sci. Int.*, 165 (2007), p. 216