Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 857 (2007) 259–265
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Comprehensive screening method for the qualitative detection of narcotics and stimulants using single step derivatisation

W. Van Thuyne *, P. Van Eenoo, F.T. Delbeke

DoCoLab, UGent, Department of Clinical Chemistry, Microbiology and Immunology, Technologiepark 30, B-9052 Ghent, Zwijnaarde, Belgium

Received 14 March 2007; accepted 19 July 2007
Available online 28 July 2007

Abstract

A selective and sensitive screening method for the detection of prohibited narcotic and stimulating agents in doping control is described and validated. This method is suitable for the detection of all narcotic agents mentioned on the World Anti-Doping Agency (WADA) doping list in addition to numerous stimulants. The analytes are extracted from urine by a combined extraction procedure using CH_2Cl_2/MeOH (9/1, v/v) and *t*-butylmethyl ether as extraction solvents at pH 9.5 and 14, respectively. Prior to GC–MS analysis the obtained residues are combined and derivatised with MSTFA. The mass spectrometer is operated in the full scan mode in the range between *m/z* 40 and 550. The obtained limits of detection (LOD) for all components included in this extensive screening method are in the range 20–500 ng/ml, which is in compliance with the requirements set by WADA. Besides narcotic and stimulating agents, this method is also capable of detecting several agents with anti-estrogenic activity and some beta-agonists. As an example, a positive identification of hydroxyl-methoxy-tamoxyfen is shown.

Keywords: GC–MS; Doping; Narcotic agents; Stimulants; Derivatisation

1. Introduction

According to the World Anti-Doping Agency (WADA) narcotic and stimulating agents can be misused in sports and are considered as doping agents [1]. Although stimulants can be analysed using LC–MS [2] at concentrations in compliance with WADA requirements of 500 ng/ml [3], screening methods for narcotics and stimulants most frequently rely on gas chromatography. Volatile and unconjugated stimulants are most commonly analysed using gas chromatography with nitrogen–phosphorus detection (NPD) [4–6], while conjugated narcotics and stimulants are analysed using GC–MS after hydrolysis of phase-II metabolites and selective derivatisation [6].

According to the criteria set by WADA, the unequivocal identification of suspicious substances should be achieved by the combination of both retention time and mass spectrometric data [7]. Suspicious results obtained from GC-NPD screening methods are only based on retention time criteria and, as a consequence, different confirmation procedures need to be developed and validated. As numerous stimulants show poor mass spectra [8] the use of a derivatising agent to detect volatile substances by GC–MS is mandatory. A reagent very often used is trifluoroacetic acid anhydride (TFAA), resulting in *N*-TFA functionalities, generating sufficient diagnostic ions to meet the WADA confirmation criteria [9].

Screening methods for the combination of conjugated narcotic agents and stimulants using GC–MS also require derivatising agents to generate the required number of three diagnostic ions characterising the detected substance. In doping control most often *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) is used creating *O*-TMS functionalities. Moreover, MSTFA is frequently combined with *N*-methylbistrifluoroacetamide (MBTFA) resulting in *N*-TFA functionalities. However, the combination of both agents does not always result in the formation of a single derivative. Analysis of beta-blocking agents, for instance, can result in multiple derivatives [6].

In addition, the use of TFA derivatising agents such as MBTFA and TFAA, while giving an excellent derivative, is pernicious for column lifetime due to decomposition of the stationary phase. This results in bad chromatography and decreased sensitivity which makes the column useless for other applications especially for the analysis of non-derivatised samples.

* Corresponding author. Tel.: +32 9 331 32 95; fax: +32 9 331 32 99.
E-mail address: wim.vanthuyne@ugent.be (W. Van Thuyne).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.07.026

The aim of this work therefore was the development of a comprehensive screening method, which combines the former screening methods using GC-NPD and GC–MS and that renders sufficient diagnostic ions to be used for confirmation purposes. In addition, it was our aim to increase productivity by avoiding frequent column replacements allowing the instrument to have a multifunctional role.

2. Experimental

2.1. Chemicals and reagents

Morphine, oxymorphone, buprenorphine and EDDP (metabolite of methadone) were purchased from Cerrilliant (Round Rock, TX, USA). Codeine and pemoline were purchased from Boehringer-Ingelheim (Brussels, Belgium). Hydromorphone, ethylmorphine, heroin (diacetylmorphine), dextromoramide, oxycodone, fentanyl, pethidine, dimethylamphetamine HCl, mephentermine sulphate, amiphenazole, phendimetrazine HCl and methadone were obtained from Sigma (Bornem, Belgium). Normethadone was obtained from Bios-Coutelier (Brussels), nalorphine (internal standard) from Janssen-Pharmaceutica (Beerse, Belgium) and pentazocine from Whintrop Laboratories (Newcastle, United Kingdom).

Bambuterol, methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDEA) and methylenedioxymethylamphetamine (MDMA) were a kind gift from the Portuguese doping control laboratory. Fencamfamine HCl, norephedrine HCl, norpseudoephedrine HCl, pseudoephedrine HCl and methamphetamine HCl were purchased from Merck (Darmstadt, Germany), pipradrol HCl from Merrell-DOW (Cincinnati, OH, USA) and amphetamine sulphate and triamterene from GlaxoSmithKline (Philadelphia, USA). Phenmetrazine and prolintane HCl were a gift from Boehringer & Sohn (Ingelheim am Rhein, Germany). Heptaminol HCl was purchased from Ets. A De Bournonville (Braine L'Alleud, Belgium), norfenfluramine HCl from Eutherapie Benelux (Brussels), ephedrine HCl from Hoechst AG (Frankfurt, Germany) and fenfluramine HCl, amineptine, amineptine C5-metabolite and fenspiride HCl from Laboratoires Servier (Orleans, France). Methylephedrine HCl was purchased from Laboratoire G.A. (Cochard, France), phentermine HCl from NV Certa Noville (Mehaigne, Belgium), nikethamide and methylphenidate from Ciba-Geigy (Groot-Bijgaarden, Belgium) and mefenorex from Produits Roche (Brussels). Chlorphentermine HCl was purchased from Tropon GmbH (Cologne, Germany). Isopropylhexedrine was purchased from Veride (Diegem, Belgium) and ethylamphetamine HCl from Will-Pharma Benelux (Brussels). Crotethamide, cropropamide, OH-bromantane, carphedone and benzylpiperazine were purchased from NMI (Pymble, Australia), fencamine from Laboratoires Miquel S.A. (Barcelona, Spain), pholedrine from Knoll AG (Ludwigshaven, Germany), fenethylline from Chemiwerk Hamburg (Germany), etamivan from Sinclair Pharmaceuticals Ltd. (Godalmings, UK) and benzoylecgonine from Lipomed (Arlesheim, Switzerland). Furfenorex and clobenzorex were obtained from Roussel Uclaf (Romainville, France), methoxyphenamine and benzphetamine from Upjohn (Kalamazoo, USA), amfepramone from Lab. Pharm. R.H. Trenker (Brussels, Belgium), dimefline from Recordate Industria Chemica & Farmaceutica (Milan, Italy), lidocaine from Astra Chemicals (Brussels, Belgium), propoxyphen from Park Davis (Bornem, Belgium) and formoterol from Novartis (Arnhem, The Netherlands). Aminogluthetimide was purchased from European Pharmacopeia (Strasbourg, France) and cyclopentamine from Eli Lilly (Brussels).

Excretion urines of the aromatase-inhibitors clomiphene, cyclofenyl, tamoxifen, anastrazole, and letrozole as well as from the stimulants prolintane, sibutramine and amfepramone were obtained after the controlled administration of a therapeutic dose and provided to the lab by other laboratories, the World Association of Anti-Doping Scientists (WAADS), the International Olympic Committee (IOC) and WADA.

MSTFA was purchased from Chem. Fabrik Karl Bucher (Waldstedt, Germany) and the enzyme preparation β-glucuronidase from *Escherichia coli* K12 was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Tertiary-butylmethylether (TBME) was purchased from Biosolve (Valkenswaard, The Netherlands), CH_2Cl_2 from Acros (Geel, Belgium), MeOH from Fisher Scientific (Loughborough, UK) and KOH, Na_2HPO_4, NaH_2PO_4, NH_3 (25%, H_2O) NH_4Cl, NaCl and Na_2SO_4 were all from Merck.

Ammonium buffer was prepared by the addition of 25% (v/v) ammonia to a saturated NH_4Cl solution until pH 9.5. The phosphate buffer (pH 7) was prepared by dissolving 7.1 g $Na_2HPO_4 \cdot 2H_2O$ and 1.4 g $NaH_2PO_4 \cdot H_2O$ in 100 ml water.

2.2. GC/MS conditions

The GC/MS analysis was carried out on an Agilent 5973 mass spectrometer (Palo Alto, CA, USA) directly coupled to an Agilent 6870 gas chromatograph equipped with a J&W-Ultra 1 column with a length of 17 m, internal diameter of 0.2 mm and a film thickness of 0.11 μm (J&W, Folsom, USA). The GC was operated in constant flow mode at a flow rate of 0.6 ml/min (linear velocity 37 cm/s). The oven temperature was as follows: 60 °C (0 min) → 90 °C/min → 100 °C (5 min) → 20 °C/min → 300 °C (3 min). Half a microliter was injected in the splitless mode. The transfer line was set at 320 °C.

The mass spectrometer was operated in the full scan mode between *m/z* 50 and 550. The electron energy of the source was set at 70 eV. The ion source and the quadrupole were set at 250 and 150 °C, respectively. The electron multiplier voltage was derived from the autotune settings and varied between 1400 and 1800 V.

2.3. Extraction

Extraction was performed with 4 ml of urine divided in aliquots of 3 and 1 ml for the extraction of the conjugated and free components, respectively.

One ml of phosphate buffer, 50 μl of β-glucuronidase and 50 μl of the internal standard nalorphine (20 μg/ml, MeOH) were added to 3 ml of urine after which the sample was hydrol-

ysed overnight at 42 °C. Extraction was performed with 5 ml of CH_2Cl_2/MeOH (9/1) after the hydrolysate was made alkaline with 0.5 ml ammonium buffer. After rolling for 20 min and centrifugation (1200*g*, 5 min) the organic layer was separated and evaporated under oxygen free nitrogen at 40 °C.

To another aliquot of 1 ml urine, 50 μl of the internal standard cyclopentamine (100 μg/ml, MeOH) and 0.5 ml KOH (5 M) were added together with 1 g NaCl and 1 ml TBME. After rolling for 1 h and centrifugation, the organic layer was added to the dried residue of the extraction performed at pH 9.5 and evaporated under oxygen free nitrogen at room temperature. The final residue of the combined extracts was derivatised with 100 μl MSTFA for 10 min at 80 °C.

2.4. Method validation

The method validation was performed according the Eurachem guidelines [10] on 10 different, randomly chosen urine samples.

To determine the limits of detection (LOD), 10 different urine samples were spiked with reference mixtures at different levels in the concentration range of 20–1000 ng/ml (20, 50, 100, 200, 250, 500 and 1000 ng/ml). The LOD was defined as the lowest concentration where a substance can be detected in all samples analysed ($n = 10$). Repeatability was assessed through the analysis of multiple samples spiked at different levels during the determination of the LOD. Selectivity and specificity were tested by the analysis of a reference mixture of numerous anabolic steroids, corticosteroids and diuretics and 10 different blank urine samples.

Confirmation thresholds for substances with a urinary threshold level according to the WADA list of prohibited substances [1] were determined by the analysis of 10 urine samples spiked at half of the urinary threshold level and at the threshold level. For each component a ratio (mean + S.D.) of the abundances of a diagnostic ion of the analyte and the internal standard was determined.

3. Results and discussion

Although the WADA technical documents for the identification of substances in doping control only recommend the use of an internal standard for quantitative confirmation procedures [7], its use in qualitative screening procedures is an indication of the effectiveness of the extraction procedure. Preliminary tests for robustness have shown that diphenylamine, a substance frequently used as internal standard for the determination of volatile stimulating agents [6], is not ideal as it seems to be extracted independently of the urinary pH as a result of the stabilising effect caused by the delocalisation of the formed charge/free electrons to the phenyl groups attached to the secondary amine function. Therefore, cyclopentamine was preferred as internal standard in this screening method. To avoid loss of volatile stimulants during the final evaporation step aqueous or methanolic solutions of HCl can be added [11]. However, reproducible results have also been reported without this step [12].

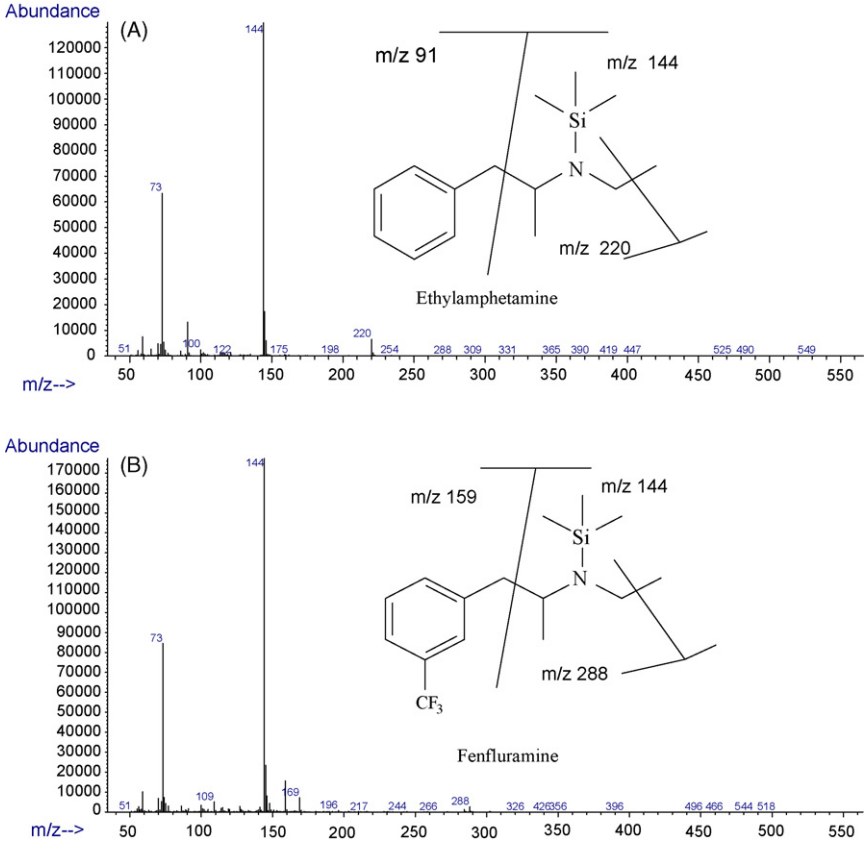

Fig. 1. Fragmentation and mass spectrum of ethylamphetamine (A) and fenfluramine (B) as their trimethylsilyl derivatives.

This method allows for the detection of more than 90 different components, including all narcotic agents from the WADA doping list and numerous stimulating agents [1]. Several substances can also be identified in urine samples by the detection of one or more metabolites. For methadone for instance, the parent compound as well as the metabolites normethadone and EDDP are monitored [13].

As several stimulants are derived from amphetamine they show an identical fragmentation pathway resulting in similar base peaks. Both ethylamphetamine and fenfluramine have a base peak of *m/z* 144 (Fig. 1). As most stimulants are very volatile, some of them co-elute in the first part of the chromatographic run. Identification of those substances can be achieved using retention time locking and the use of minor diagnostic ions. As an example, Fig. 2 shows the ion chromatogram (*m/z* 144) for ethylamphetamine and fenfluramine which are partially coeluting and the ion chromatograms of the respective minor ions *m/z* 159 and 220 which are separated at concentrations equal to 500 ng/ml.

Besides these categories of doping agents, two beta-agonists, formoterol and bambuterol, were also included in this screening method.

This method is also capable of detecting several metabolites or parent compounds of agents with anti-estrogenic activity such as aminogluthetimide, clomiphene, cyclofenyl, anastrazole, letrozole and tamoxifen (Fig. 3). Using this full scan screening procedure, positive screening results could be obtained for hydroxy-methoxy-tamoxifen (Fig. 4), the main metabolite of tamoxifen, in a WADA PT-test sample while results obtained in the selected ion monitoring mode after liquid/liquid extraction with diethylether were not conclusive.

The GC relative retention times calculated against the internal standard nalorphine and diagnostic ions used for compound identification are given in Table 1. A maximal relative deviation in intensities of 20% of the monitored ions was used as a qualitative criterion for the relative abundances compared to a quality control sample spiked with all compounds at a concentration equal to the MRPL. Additionally, a maximal deviation of 1% in relative retention time was used as second criterion.

The validation procedure of this method was performed using 10 different negative control urine samples (pH 5.8–7.2; s.g. 1.012–1.032 g/ml). Simultaneously, prolintane excretion urines were analysed. The detection of the four main metabolites of prolintane [14] (Fig. 3), all excreted as glucuronides, was used as indication of the effectiveness of the enzymatic hydrolysis. Quality control samples analysed simultaneously with real urine samples are also spiked to prolintane excretion urine to prove the efficacy of the enzymatic hydrolysis in each batch. In addition, the mono-TMS derivatives of the testosterone metabolites androsterone and etiocholanolone can also be used as these should be detected in all samples analysed. All stimulants, except carphedon, were detected at levels far below the WADA MRPL level of 500 ng/ml. LODs for narcotics were all at or below the WADA MRPL of 200 ng/ml. Although at present no MRPL level for beta-agonists is set by WADA, this method allows for the detection of bambuterol and formoterol at levels down to 20 ng/ml.

Besides the determination of the LOD, selectivity and specificity were also tested during method validation. An analytical method is selective if it can distinguish the analytes from other substances belonging to the same category. Injection of reference mixtures of other doping substances (i.e. anabolic steroids, corticosteroids and diuretics) tested selectivity. According to Verwaal et al. [15] the concentration of these related agents should at least be twice the LOD of the determined components. No interferences were noticed at the retention time of the analytes or at the retention time of the internal standard. Selectivity was tested by the analysis of the 10 different negative urines used to determine the LOD of the analytes. No interferences were observed that could hamper the positive identification of the analytes. Hence, the analytical method was selective and specific.

This method comprises several substances with a urinary threshold level according to WADA criteria [1]. These substances include morphine, ephedrine, cathine (norpseudoephedrine) and methylephedrine. In order to avoid unnec-

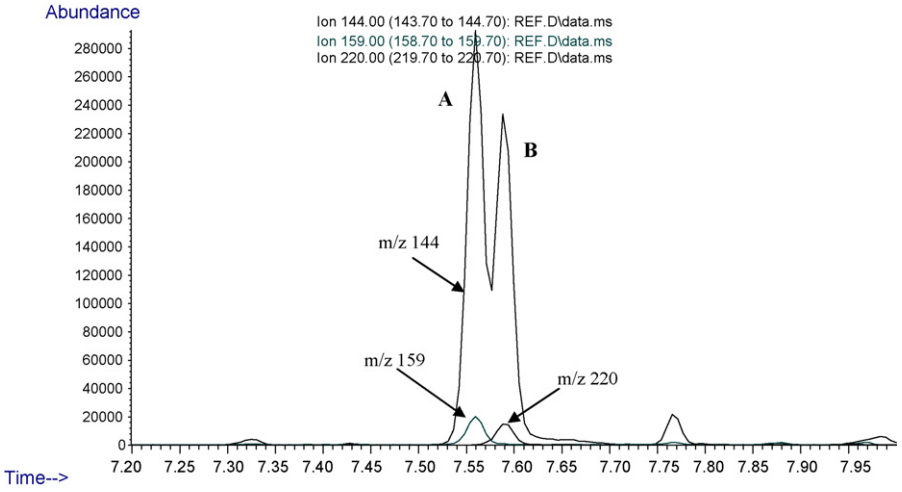

Fig. 2. Identification of ethylamphetamine (A) and fenfluramine (B) using minor diagnostic ions *m/z* 159 and 220.

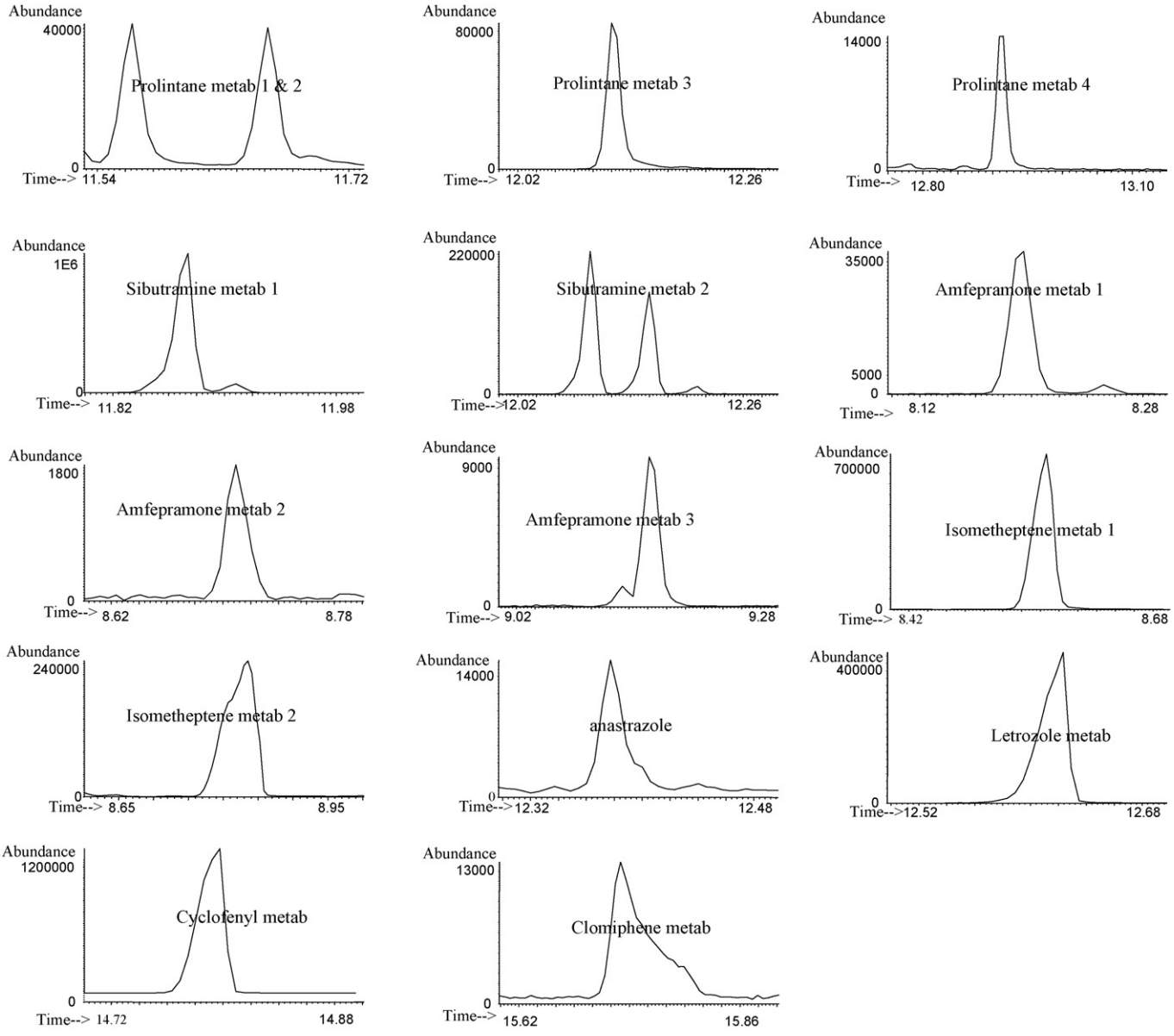

Fig. 3. Ion traces of the most abundant ions of components detected via administration urines.

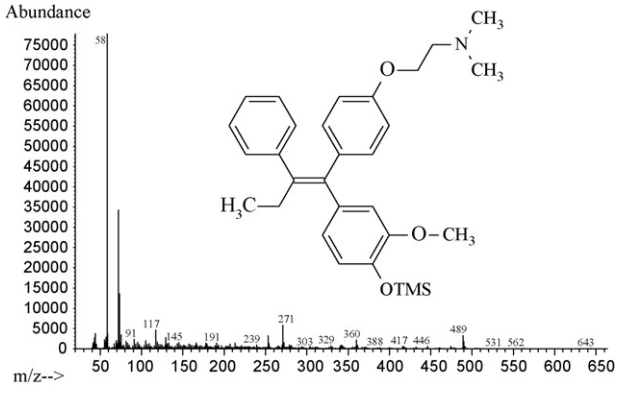

Fig. 4. Positive screening results for trimethylsilyl-hydroxy-methoxy-tamoxifen.

essary confirmation procedures of suspicious peaks with abundances far below the respective threshold levels, "confirmation limits" were introduced above which a sample will be subjected to quantitative analysis.

The confirmation procedure for morphine is identical to this screening method excepting the omission of the extraction at pH 14 [16]. As the extraction recovery of morphine-glucuronide at this pH and the prevalence of unconjugated morphine are negligible [17], screening results can be quantified using the same calibration curve of the confirmation procedure for morphine. A validation procedure, also testing the completeness of the enzymatic hydrolysis procedure, conducted on 10 samples spiked with morphine-3-glucuronide at 0.5 μg/ml (morphine equivalent) pointed out that a confirmation threshold at this level can be used without the risk of false negative results (mean: 0.53 μg/ml; S.D.: 0.02 μg/ml).

Table 1
Retention times (RT), relative retention times (RRT), diagnostic ions and LODs of investigated components

Component	RT (min)	RRT	Diagnostic ions (*m*/*z*)	LOD (ng/ml)
Dimethylamphetamine[a]	3.48	0.249	72; 91; 148	50
Mephentermine[a]	3.78	0.270	72; 91; 148	50
Cyclopentamine	4.53	0.324	130; 116; 198	IS
Amphetamine	4.95	0.354	91; 116; 192	20
Norfenfluramine	5.11	0.365	116; 159; 260	50
Methylphenidate	6.20	0.443	56; 84; 91	100
Isopropylhexedrine	6.36	0.454	116; 130; 212	50
Phentermine	6.51	0.465	114; 130; 206	20
Metamphetamine	6.58	0.470	91; 130; 206	50
Heptaminol	7.22	0.516	116; 131; 274	50
Methylephedrine	7.27	0.519	72; 102; 236	20
Phendimetrazine[a]	7.42	0.530	57; 85; 191	50
Fenfluramine	7.66	0.547	144; 159; 288	20
Ethylamphetamine	7.69	0.549	91; 144; 220	20
Diethylpropion[a]	7.86	0.561	72; 100; 144	100
Cathine	7.96	0.569	116; 147; 280	50
Nikethamide[a]	8.04	0.574	78; 106; 177	100
Norephedrine	8.05	0.575	116; 147; 280	50
Amfepramone metab 1	8.36	0.597	100; 221; 264	EU
Methoxyphenamine	8.48	0.606	91; 130; 236	50
Mefenorex[a]	8.57	0.612	56; 91; 120	50
Chlorphentermine	8.59	0.613	130; 114; 240	100
Isometheptene metab 1	8.59	0.614	115; 130; 286	EU
Ephedrine	8.64	0.617	130; 147; 249	50
Pseudoephedrine	8.70	0.622	130; 147; 249	50
Isometheptene metab 2	8.75	0.625	130; 286; 301	EU
Amfepramone metab 2	8.84	0.632	144; 234;243	EU
Prolintane[a]	8.98	0.641	91; 126; 127	100
MDA	9.02	0.645	116; 135; 236	250
Phenmetrazine	9.17	0.655	100; 234; 249	50
Crothetamide[a]	9.28	0.663	86; 154; 181	20
Benzylpiperazine	9.31	0.665	102; 157; 248	200
Amfepramone metab 3	9.32	0.666	144; 207; 308	EU
Furfenorex[a]	9.32	0.666	53; 81; 138	20
Fencamfamine[a]	9.33	0.667	98; 215; 186	50
MDMA	9.56	0.683	73; 130; 250	50
Cropropamide[a]	9.63	0.688	84; 100; 168	20
Pholedrine	9.78	0.699	130; 179; 294	100
Pethidine[a]	9.80	0.701	172; 218; 247	50
Lidocaine	9.91	0.708	86; 220; 235	20
MDEA	10.15	0.726	135; 144; 264	50
Benzphetamine[a]	10.33	0.738	65; 91; 148	100
Clobenzorex[a]	11.00	0.786	125; 168; 170	200
Ethamivan	11.08	0.792	193; 223; 295	20
Pemoline	11.29	0.807	163; 178; 392	50
Carphedon	11.40	0.815	104; 272; 257	500
EDDP	11.44	0.817	220; 262; 277	200
Prolintane metabolite 1	11.59	0.828	117; 184; 304	EU
Prolintane metabolite 2	11.68	0.835	117; 184; 304	EU
Normethadone[a]	11.71	0.837	58; 224; 264	20
Pipradrol	11.90	0.851	56; 84; 239	20
Methadone[a]	11.92	0.852	72; 223; 294	100
Sibutramine metab 1	11.97	0.855	102; 158; 238	EU
Propoxyphene[a]	12.12	0.866	58; 91; 172	20
Prolintane metabolite 3	12.15	0.868	138; 228; 304	EU
Sibutramine metabolite 2	12.18	0.870	156; 246; 376	EU
Benzoylecgonine	12.47	0.891	82; 240; 361	200
Anastrazole	12.47	0.891	209; 224; 293	EU
Letrozole metabolite	12.50	0.893	190; 217; 291	EU
Pentazocine	12.50	0.893	245; 289; 357	50
Aminogluthetimide	12.85	0.918	204; 219: 361	200
Prolintane metabolite 4	12.94	0.925	140; 179; 322	EU
Codeine	13.23	0.945	178; 234; 371	20

Table 1 (*Continued*)

Component	RT (min)	RRT	Diagnostic ions (*m/z*)	LOD (ng/ml)
5-OH-pentoxyphilline	13.30	0.951	237; 337; 352	50
Ethylmorphine	13.38	0.956	192; 357; 385	20
Fenspiride	13.40	0.957	105; 241; 317	20
Hydromorphone	13.45	0.961	234; 429; 414	20
Morphine	13.52	0.966	236; 414; 429	50
Oxycodone	13.65	0.976	340; 372; 387	20
Bambuterol	13.74	0.981	72; 86; 354	20
Heroin[a]	13.86	0.990	268; 327; 369	200
Oxymorphone	13.87	0.991	287; 430; 445	200
OH-bromantane	13.92	0.994	91; 393; 395	50
Nalorphine	14.00	1.000	260; 414; 455	IS
Amineptine metabolite	14.04	1.003	115; 178; 192	100
Dimefline[a]	14.07	1.005	163; 279; 323	50
Fentanyl[a]	14.22	1.016	146; 189; 245	20
Fenethylline[a]	14.54	1.039	91; 207; 250	100
Triamterene	14.60	1.043	382; 454; 469	20
Amineptine	14.80	1.057	115; 192; 218	100
Cyclofenyl metabolite	14.85	1.061	343; 422; 512	EU
Formoterol	14.95	1.068	265; 349; 383	20
Dextromoramide[a]	14.97	1.070	100; 128; 265	50
Tamoxifen metabolite	15.30	1.093	58; 72; 489	EU
Clomiphene metabolite	15.72	1.123	58; 86; 100	EU
Buprenorphine	16.75	1.197	450; 524; 539	20

EU: Excretion urine; IS: internal standard.
[a] Underivatised.

As the confirmation procedure for ephedrines is performed at pH 14 and these substances also show a substantial recovery at pH 9.5, a different approach is needed. Using the same safety margin of 50%, avoiding the possibility of false negative screening results, suspicious cathine screening results should be confirmed if the ratio of *m/z* 116 of cathine to *m/z* 130 of the internal standard is higher than 0.5 (mean: 0.44; S.D.: 0.08), methylephedrine if the ratio of *m/z* 72 to 130 is higher than 2 (mean: 2.00; S.D.: 0.16) and ephedrine if the ratio of *m/z* 130 of ephedrine to *m/z* 130 of the internal standard is higher than 2 (mean: 2.07; S.D.: 0.21).

4. Conclusion

A comprehensive screening method was developed which combines the former screening methods for volatile stimulants and conjugated stimulants and narcotics. This method, which is capable of detecting more than 90 components, only requires derivatisation with MSTFA resulting in reduced analysis time and an increased column lifetime of approximately 1000 injections. In contrast to former NPD methods, this method provides mass spectral data reducing the number of unnecessary confirmations. The sensitivity of this method allowed for full scan mass spectrometry identification at very low concentrations. In order to reduce the number of confirmations of substances with a threshold level, confirmation limits were also introduced for substances with a urinary threshold level.

Acknowledgements

The authors wish to thank the Flemish Ministry of Culture, Youth, Sport and Brussels for financial support (WVT, PVE). The technical assistance of W. Van Gansbeke is gratefully acknowledged.

References

[1] WADA, The World Anti-Doping Code, The 2007 Prohibited List, 2007.
[2] K. Deventer, P. Van Eenoo, F.T. Delbeke, Rapid Commun. Mass Spectrom. 20 (2006) 877.
[3] WADA, Technical Document: Minimum Required Performance Limits for Detection of Prohibited Substances: TD2004MRPL, 2004.
[4] D.S. Lho, H.S. Shin, B.K. Kang, J. Park, J. Anal. Toxicol. 14 (1990) 73.
[5] P.J. van der Merwe, H.S.L. Kruger, J.W. Pieterse, M.J. Pretorius, N.J. de Cock, in: W. Schanzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.), 18th Cologne Workshop on Dope Analysis, Sport & Buch Straub, Cologne, 2000, p. 245.
[6] P. Hemmersbach, R. de la Torre, J. Chromatogr. B 687 (1996) 221.
[7] WADA, Technical Document TD2003IDCR, Identification Criteria for Qualitative Assays Incorporating Chromatography and Mass Spectrometry, Version 1.2., 2003.
[8] E. Änggard, A. Hankey, Acta Chem. Scand. 23 (1969) 3110.
[9] W.C. Thompson, A. Dasgupta, Clin. Chem. 40 (1994) 1703.
[10] Eurachem, The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics, LGC Ltd., Teddington, UK, 1998.
[11] T. Kraemer, H.H. Maurer, J. Chromatogr. B 713 (1998) 163.
[12] P. Dallakian, H. Budzikiewicz, H. Brzezinka, J. Anal. Toxicol. 20 (1996) 255.
[13] D.G. Wilkins, P.R. Nagasawa, S.P. Gygi, R.L. Foltz, D.E. Rollins, J. Anal. Toxicol. 20 (1996) 355.
[14] M. Neugebauer, G. Rucker, Arch. Pharm. 324 (1991) 396.
[15] W. Verwaal, M. van Bavel, A. Boot, J. Bravenboer, F. de Goei, C. Maas, A. van de Putten, De ware(n) Chemicus 26 (1996) 25.
[16] W. Van Thuyne, P. Van Eenoo, F.T. Delbeke, J. Chromatogr. B-Anal. Technol. Biomed. Life Sci. 785 (2003) 245.
[17] R.W. Romberg, L. Lee, J. Anal. Toxicol. 19 (1995) 157.