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Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry

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Abstract

A liquid chromatographic procedure with electrospray ionization tandem mass spectrometric detection has been developed and validated for LSD and iso-LSD determination. A one-step liquid–liquid extraction on 1 ml blood or urine was used. The lower limit for quantitative determination was 0.02 μg/l for LSD and iso-LSD. The analytical procedure has been applied in two positive cases (case 1: LSD=0.31 μg/l, iso-LSD=0.27 μg/l in plasma and LSD=1.30 μg/l, iso-LSD=0.82 μg/l in urine; case 2: LSD=0.24 μg/l, iso-LSD=0.6 μg/l in urine). LSD metabolism was investigated using MS–MS neutral loss monitoring for the screening of potential metabolites. The main metabolite was 2-oxo-3-hydroxy-LSD (O–H–LSD) present in urine at the concentrations of 2.5 μg/l and 6.6 μg/l, respectively, for case 1 and 2, and was not present in plasma. Nor-LSD was also found in urine at 0.15 and 0.01 μg/l levels. Nor-iso-LSD, lysergic acid ethylamide (LAE), trioxylated-LSD, lysergic acid ethyl-2-hydroxyethylamide (LEO) and 13 and 14-hydroxy-LSD and their glucuronide conjugates were detected in urine using specific MS–MS transitions.

Keywords: Lysergic acid diethylamide

1. Introduction

Lysergic acid diethylamide (LSD) is a semi-synthetic compound derived from ergot alkaloids. In illicit preparations, stereoisomers LSD and iso-LSD are formed. LSD is one of the most potent psychotropic drugs, only few micrograms (50–100) are need for pharmacological effects. Iso-LSD is not psychoactive but is a marker of LSD use. LSD is extensively metabolized and only a small fraction is found unchanged in biological fluids. Some metabolites [1–8] have been already identified in human biological fluids, others have been only detected during ''in vitro'' studies. The detection of very low concentrations of LSD and its metabolites require the development of specific and sensitive analytical methods. Several gas chromatographic mass spectrometric methods [9–15] have been developed for LSD analysis. These methods provide specificity and high selectivity but need time-consuming extraction, purification and derivatization procedures. Recently, some authors [15–20] have proposed liquid chroma-

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tography–mass spectrometry (LC–MS) methods for its determination. Electrospray ionization is a process providing a protonated molecular ion. In tandem mass spectrometry, this ion selected by the first quadrupole Q1, is fragmented in the collision cell Q2 of the mass spectrometer and produced fragment ions are monitored by the last quadrupole Q3. For quantitative analysis, selections of protonated molecular ion/fragment ion transitions called multiple reaction monitoring (MRM) for known compounds can confer very high sensitivity to methods.

In this paper, a rapid, sensitive and specific method is described for quantitation of LSD and metabolites using specific transitions for parent drug and known metabolites. For the identification of LSD metabolites, neutral loss scan and product ion scan using the calculated molecular mass of each known or putative metabolite were used.

2. Materials and methods

2.1. Materials

All solvents (HPLC grade) and others chemicals (formic acid, ammonium formate, ammonia) were provided by Merck (Darmstadt, Germany).

Stock solutions (25 mg/l in acetonitrile) of LSD, iso-LSD, nor-LSD, nor-iso-LSD, 2-oxo-3-hydroxy-LSD and trideuterated analog of LSD were obtained from Radian (Austin, TX, USA).

Working standards (1, 10, 100 μg/l) and internal standard (5 μg/l) solutions were prepared with deionized water and stored at −4°C.

Drug-free urine, plasma and blood were collected from healthy subjects.

For the validation of the method, the samples were prepared by adding the working standards into drug-free urine to obtain a concentration of each compound at 0–4 μg/l for the linearity study, at 0.02, 0.2 and 2 μg/l for precision and recoveries.

2.2. Instrumentation

Analysis was performed on a triple quadrupole API 300 Perkin-Elmer SCIEX (Thornhill, Canada) mass spectrometer equipped with an atmospheric pressure ionization source via an ionspray interface.

For liquid chromatography two micropumps and an autosampler series 200 Perkin-Elmer were used. Drugs were separated on a Spherisorb 5 RP 8S (100×2.1 mm, I.D., 5 μm, Applied Biosystems Perkin-Elmer, Les Ulis, France).The mobile phase was a mixture of water (40%) and acetonitrile (60%) containing 0.1% HCOOH and 2 m*M* of $HCOONH_4$. Solvent flow was set at 400 μl/min.

Using electrospray tandem mass spectrometry, LSD and metabolites spectra were obtained in positive ionization mode. The electrospray evaporation process produced for each compound an abundant protonated molecular ion (MH^+). For each analyte, this ion is selected in the first quadrupole Q1 and fragmented in the collision cell Q2 with nitrogen gas. The product ion spectra are obtained in the second quadrupole Q3.

For quantitative determinations, one resulting product ion for each compound of interest is monitored in Q3. This operating mode called MRM provides less specificity than a complete MS–MS spectrum but increases specificity over a single stage of mass spectrometric analysis. The MRM experiments used for detection and quantitation are reported in Table 1. The same transition was used for LSD and iso-LSD. Mass spectrometer setting was optimized for LSD to give optimum ion yield. Electrospray voltage was adjusted at 5.2 kV, the ion extraction voltage at 60 V and the fragmentation energy at 27 eV.The turbo temperature was 350°C and the turbo heater gas flow of nitrogen was 7 l/min. Nitrogen was also used as nebulizer, curtain and collision gas. Collision gas flow setting was 2 (instrument units). Data were processed using the API 300 standard software MacQuan from PE Sciex (Foster City, CA, USA).

For metabolite identification, other MS–MS experiments were used. Metabolites generally disso-

Table 1
MRM transitions for quantification

Compound	Protonated molecular ion (a.m.u.)	Fragment ion (a.m.u.)
LSD	324.2	223.1
Iso-LSD	324.2	223.1
Nor-LSD	310.2	209.1
2-Oxo-3-hydroxy-LSD	356.2	338.1
Trideuterated LSD	327.2	226.1

Table 2
Intra-day and inter-day precisions for LSD and iso-LSD

	LSD			Iso-LSD		
Spiked conc. (μg/l)	0.02	0.2	2	0.02	0.2	2
Intra-day precision n=6						
Mean conc.	0.0195	0.194	1.94	0.0231	0.190	2.094
±s	0.0012	0.0056	0.078	0.0025	0.0079	0.109
C.V. (%)	6.3	2.9	4.0	11.1	4.2	5.2
Inter-day precision n=6						
Mean conc.	0.0187	0.206	2.11	0.0205	0.191	2.19
±s	0.0012	0.0056	0.112	0.0014	0.0177	0.022
C.V. (%)	6.5	2.7	5.3	6.7	9.3	6.8

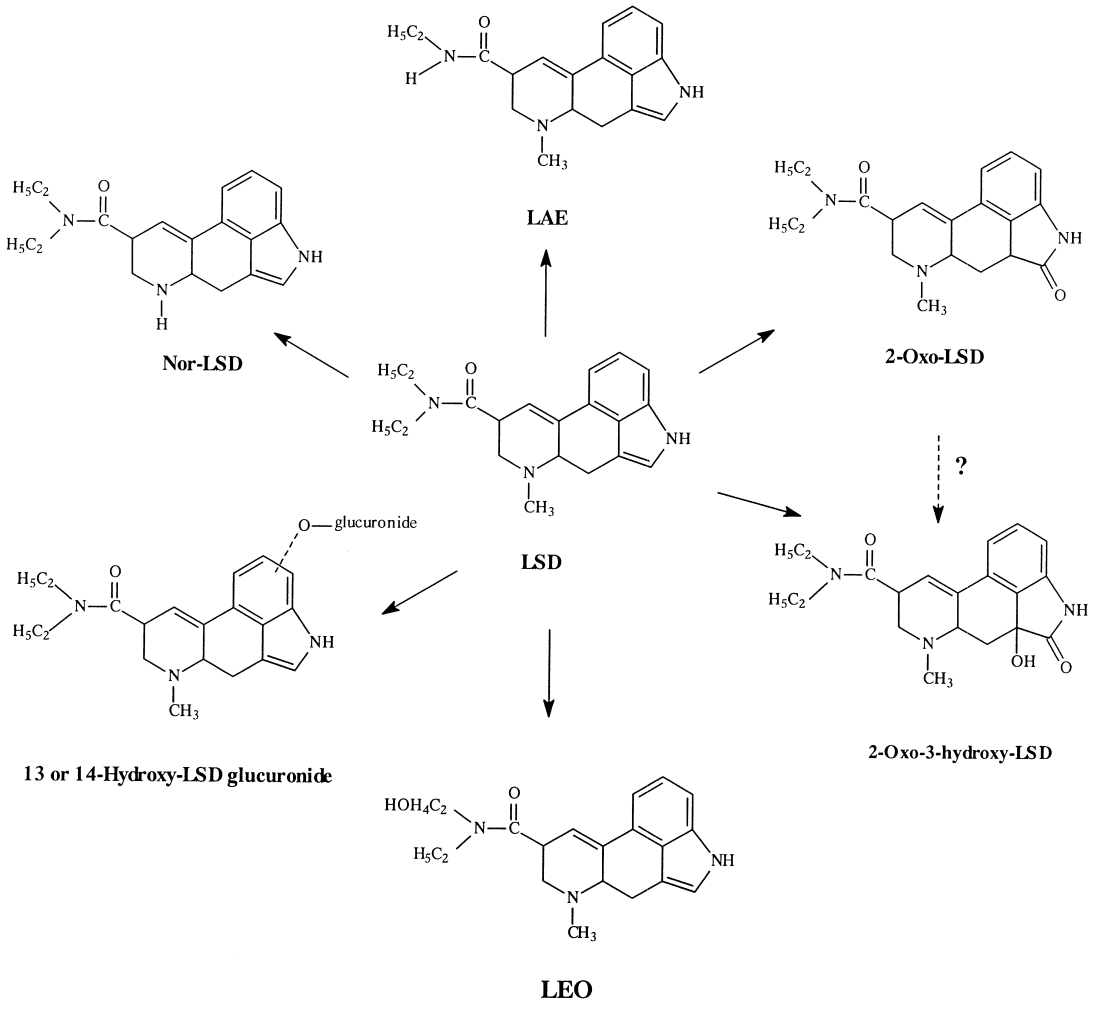

Fig. 1. Metabolism pathway for LSD.

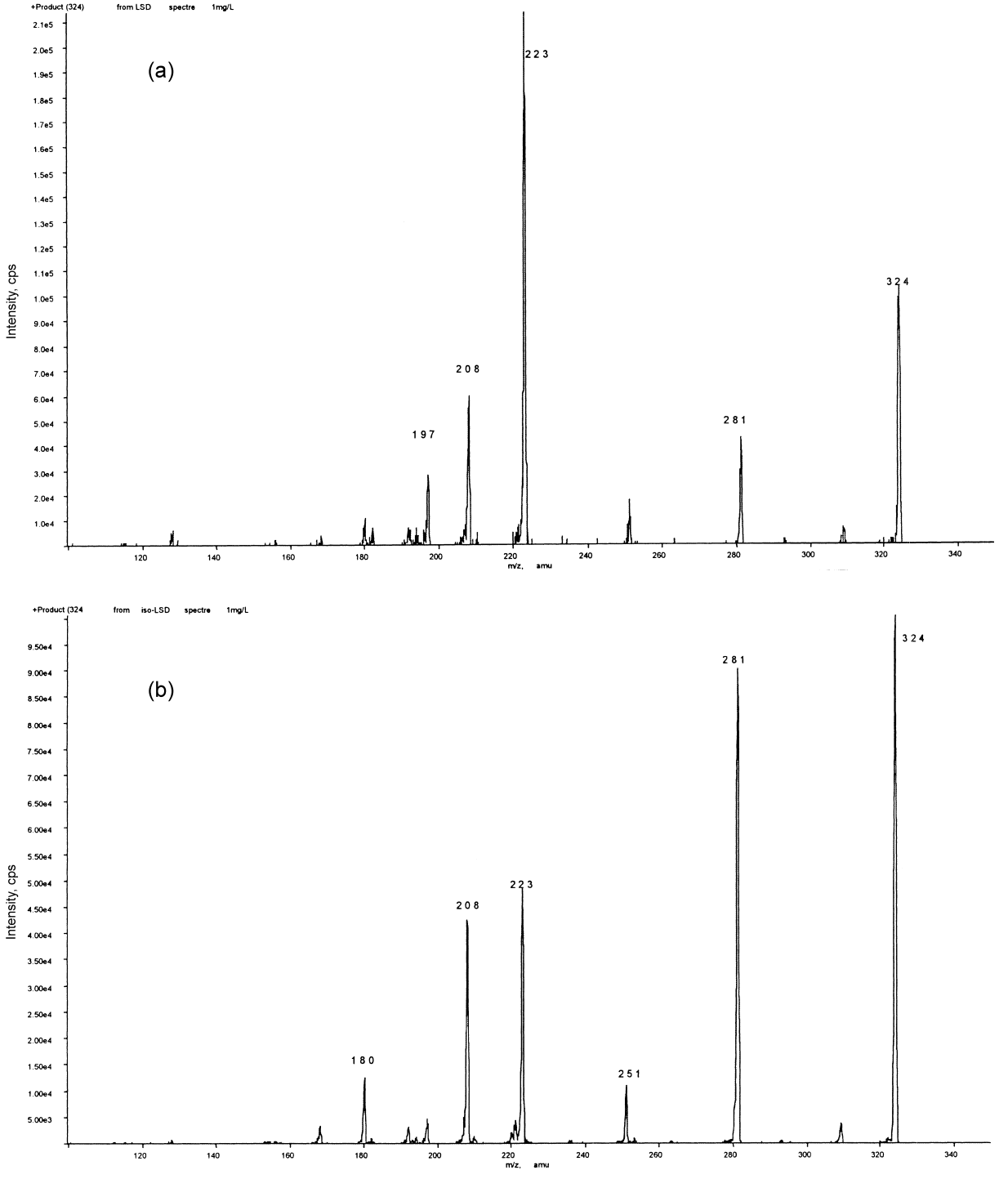

Fig. 2. (a) LSD product ion scan. (b) Iso-LSD product ion scan.

ciate by losses of the same typical neutral fragments associated with a functional group or substructure of the parent drug. In order to screen potential metabolites, neutral loss scan of specific fragment was operated. When sensitivity was not sufficient with this mode, calculation of the molecular mass was done for every possible metabolite and several (2 or 3) specific transitions were monitored for each compound.

2.3. Sample preparation

To 1 ml of urine, plasma or blood were added 20 μl of a solution of internal standard (trideuterated LSD) at 5 μg/l, 500 μl of ammonia buffer (1 *M*, pH 9) and 6 ml of diethylether. After agitation on a rotative shaker for 10 min and centrifugation, the organic phase was evaporated to dryness. The residue was dissolved in 100 μl of water–acetonitrile (50/50, V/V) and 25 μl were injected into the LC–MS–MS.

3. Results and discussion

3.1. Validation

Validation was done on LSD and iso-LSD only. Linearity, limit of quantitation, intra-day and inter-day precisions were studied with urine. For accuracy, drug-free urine, plasma and blood were used. Validation of the procedure is reported in Table 2.

The linearity was tested at 10 levels of concentration: 0, 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 1, 2, 4 μg/l. The procedure exhibits linearity for LSD and iso-LSD. Correlation coefficients greater than 0.998 have been obtained for the calibration curves of each analyte.

The limit of quantitation established using drug-free samples to which LSD and iso-LSD were added, was obtained for concentrations of 0.02 μg/l in analytes. Six extractions of the same spiked urinary sample were analysed. The quantification limit was found to be 0.02 μg/l for LSD and iso-LSD based

Table 3
LSD and metabolites in urine for case 1

Identified compounds	Neutral loss *m/z*	Selected transitions *m/z*	Retention times (min)	Intensity arbitrary units (cps)
LSD	43	324/281	4.3	36 000 (1.3 μg/l)
	73	324/251		
	101	324/223		
	101+15	324/208		
Iso-LSD	43	324/281	6.1	2000 (0.82 μg/l)
	73	324/251		
	101	324/223		
	101+15	324/208		
2-Oxo-3-hydroxy-LSD	18	356/338	1.9	11 500 (2.5 μg/l)
	73+18	356/265		
	101+18	356/237		
Nor-LSD	73	310/237	4.0	1300 (0.15 μg/l)
	101	310/209		
Nor-iso-LSD	73	310/237	4.9	200
	101	310/209		
HO–LSD glucuronides	176	516/340	0.85 and 1.0	1200–1900
Tri-oxo LSD	101+18	372/253	0.9	5500
	73+43+18	372/238		
LAE	43	296/253	3.4	2000
	73	296/223		
	73+15	296/208		
LEO	101+16	340/223	2.7	350
	101	340/239	2.3	500

on a coefficient of variation lower than 20% and recoveries comprised between 80 and 100%.

Precision data were determined by replicate analyses of a pooled drug-free urine spiked at three levels of concentration (0.02, 0.2, 2 μg/l). The intra-day C.Vs were calculated from six replicate analyses on a single day and inter-day C.Vs from a single analysis on six different days. The mean values, standard deviations and variation coefficients are shown in Table 2.

Accuracy was tested on six different urine, plasma and blood samples. All matrix samples were spiked at 0.02, 0.2, 2 μg/l levels of LSD and iso-LSD. The recoveries were calculated by comparing theoretical concentrations to those measured using calibration curves. Results between 88% and 110% were obtained for the compounds.

3.2. Metabolism identification: Case reports

3.2.1. Case 1

Blood and urine samples were obtained from a 21-year-old male admitted in the emergency unit of the hospital 4 h after ingestion of LSD blotter.

Quantitative analysis: Urinary screening of illicit drugs were operated by LC–MS–MS methods. Opiates, amphetamines and cocaine detections were negative. Low levels of cannabis (THCCOOH 30 μg/l, THC–OH 5 μg/l, THC 3 μg/l) were detected. LSD and iso-LSD levels in urine were 1.3 μg/l and

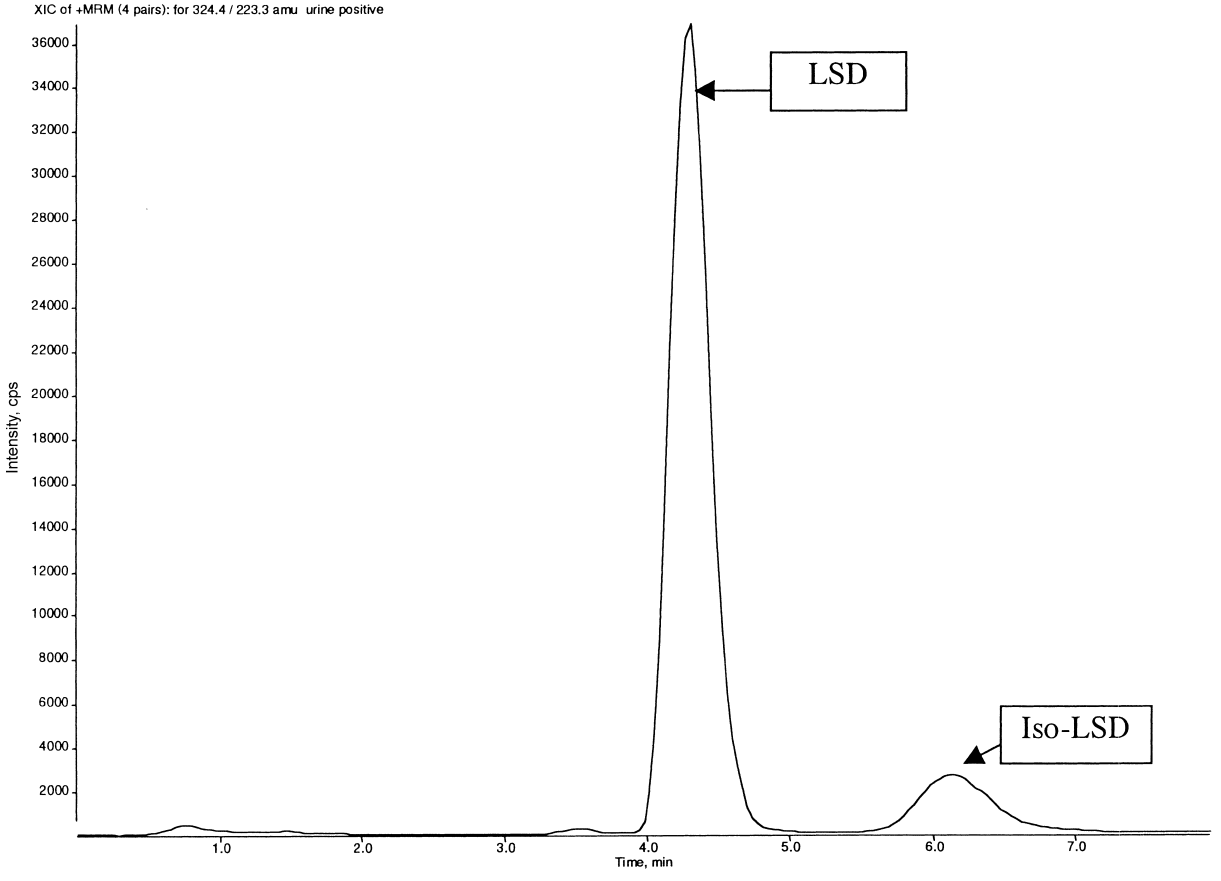

Fig. 3. LSD and iso-LSD determination in a LSD-positive urine sample.

0.82 μg/l, respectively, and 0.31 μg/l and 0.27 μg/l in plasma.

3.2.2. Case 2

Urine was obtained from a 22-year-old male, 7 h after LSD ingestion.

Quantitative analysis: Urinary screening of illicit drugs were done by immunologic methods. Opiates, amphetamines and cocaine detections were negative. Cannabis detection was positive (>50 μg/l) but quantitation by LC–MS–MS was not done. Urinary LSD and iso-LSD levels were 0.24 μg/l and 0.6 μg/l, respectively.

3.3. Metabolism exploration

Because LSD is rapidly and extensively metabolized, there may be interest in identifying its metabolites for increasing time during which its absorption can be detected. Metabolites are often obtained after *N*-dealkylation and/or oxidation processes and generally retain a large portion of the original compound structure. This may be helpful for their detection using tandem mass spectrometry. The metabolism pathway of LSD and iso-LSD is presented in Fig. 1.

The specific fragmentations of LSD and iso-LSD have been studied using their product ion spectra (Fig. 2a, b). They dissociate by loss of neutral

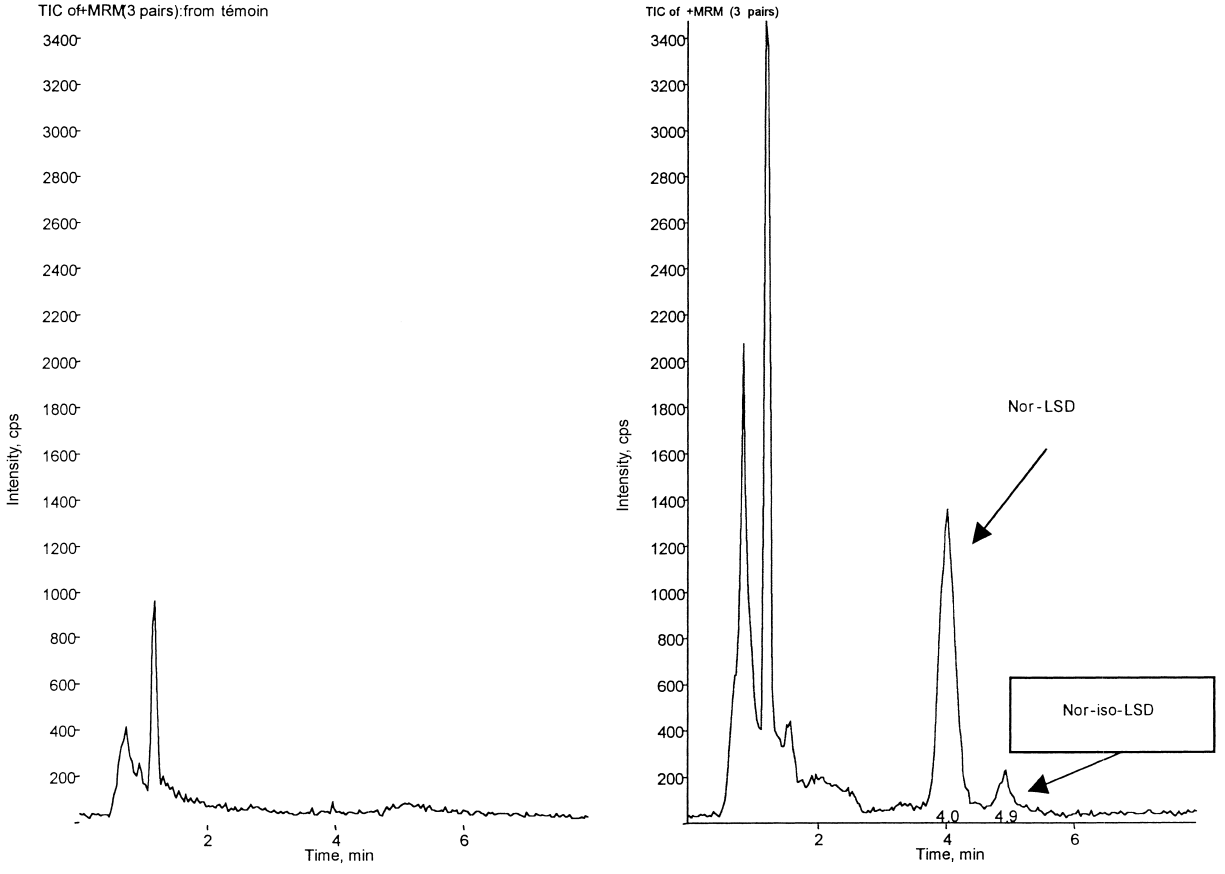

Fig. 4. Nor-LSD and nor-iso-LSD detection in a drug-free (left) and in a positive LSD urine sample.

fragments of $m/z=101$ (diethylamide), 73 (diethylamine), 43 ($CH_2=N-CH_3$) or 116 (73+43). Neutral loss MS–MS experiments for losses of 101 or 43 were used to screen some possible metabolites. Using these modes, only LSD, iso-LSD and nor-LSD were detectable. In order to obtain a better sensitivity for other compounds, calculation of molecular mass of potential metabolites was done, the corresponding protonated molecular ion was selected in Q1, fragmented in Q2 and several specific ions were monitored in Q3 (Table 3).

Metabolites were checked in urine and in plasma when possible. Positive-LSD (shown in Fig. 3) urine and drug-free urine were analysed simultaneously using the described method for each possible metabolite identification.

3.4. Metabolization via N-dealkylation compounds

3.4.1. Identification of nor-LSD and nor-iso-LSD ($MH^+=310$)

This compounds have already been described [3]. Calculation of its protonated molecular ion gives a 310 m/z value. The major product ions correspond to loss of the diethylamide group (MH^+-101) and loss of the diethylamine group (MH^+-73) from the protonated parent ion. MRM transitions 310/209 and 310/237 were checked. Compounds corresponding to nor-LSD and nor-iso-LSD were detected at retention times of 4.0 and 4.9 min, respectively (Fig. 4). Identification was subsequently proven using authentic nor-LSD and nor-iso-LSD. Nor-LSD was quantified using trideuterated LSD as internal stan-

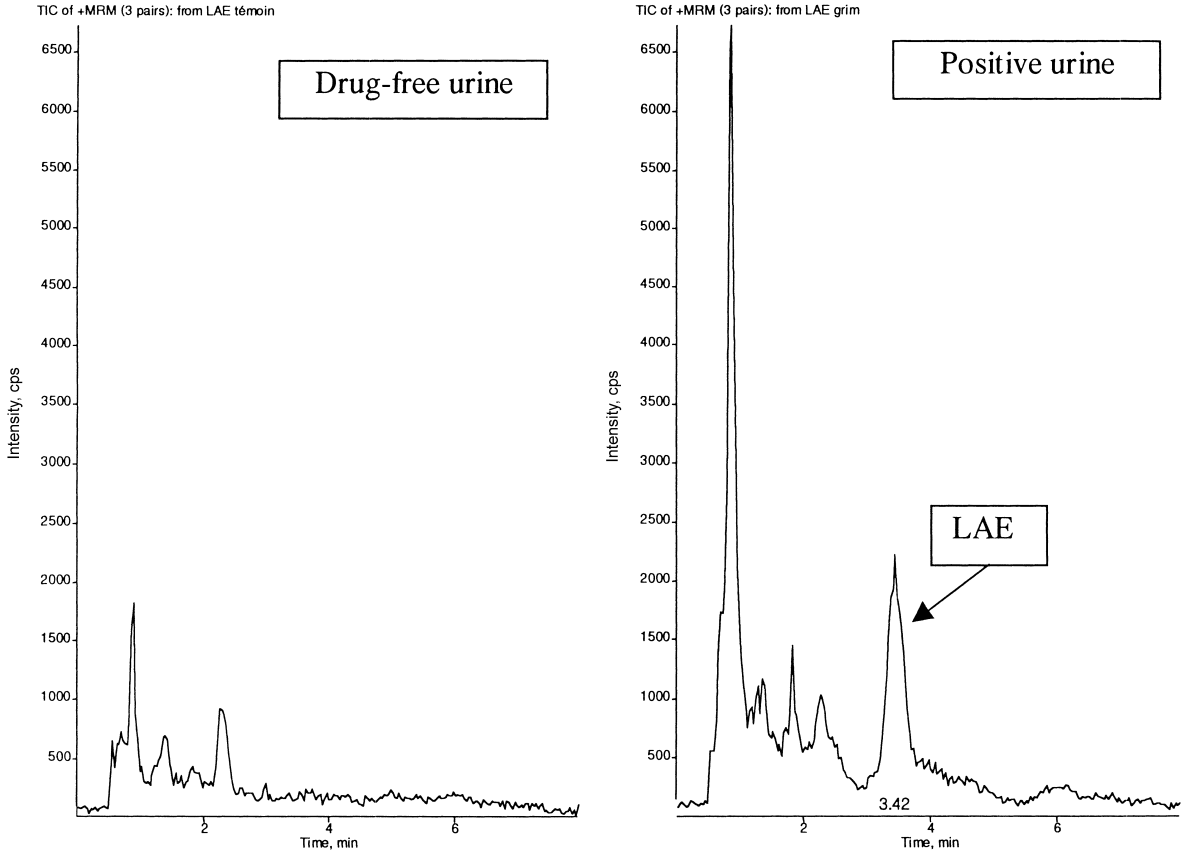

Fig. 5. Lysergic acid ethylamide (LAE) detection in a drug-free (left) and in a positive LSD urine sample.

dard, 0.15 μg/l and 0.01 μg/l were measured in case 1 and 2, respectively.

3.4.2. Identification of lysergic acid ethylamide (LAE) (MH^+=296)

LAE was determined to be the major human liver metabolite of LSD in vitro [2].This compound is not commercially available. Its protonated molecular ion was calculated at m/z=296. Three possible fragment ions m/z 253 (MH^+–CH_2=N–CH_3=43), m/z 223 (MH^+–ethylamide=loss of branched side chain=101) and m/z 208 (MH^+–ethylamide–CH3=116) arising from the parent protonated molecular ion (MH^+) were selected into the second quadrupole. The total MRM chromatogram (296/223, 296/253 and 296/208) displays two compounds at retention times of 0.9 and 3.4 min, but the last one only (3.4 min) possesses the three selected product ions and corresponds probably to LAE (Fig. 5).

3.5. Metabolization via oxidation and hydroxylation compounds

3.5.1. Identification of 2-oxo-3-hydroxy-LSD (O–H–LSD) (MH^+=356)

Recently, O–H–LSD has been found in urine of LSD users [3] in much higher concentration than LSD itself. This major metabolite of LSD was monitored for the purpose of identification by the presence of its product ions m/z 338 (MH^+−18=H_2O), m/z 265 (MH^+−91=diethylamine and H_2O) and m/z 237 (MH^+−119=diethylamide and H_2O).The retention time for the compound shown in Fig. 6 was 1.9 min and corresponded to commercial O–H–LSD. Quantitation in urine (2.5 μg/l and 6.6

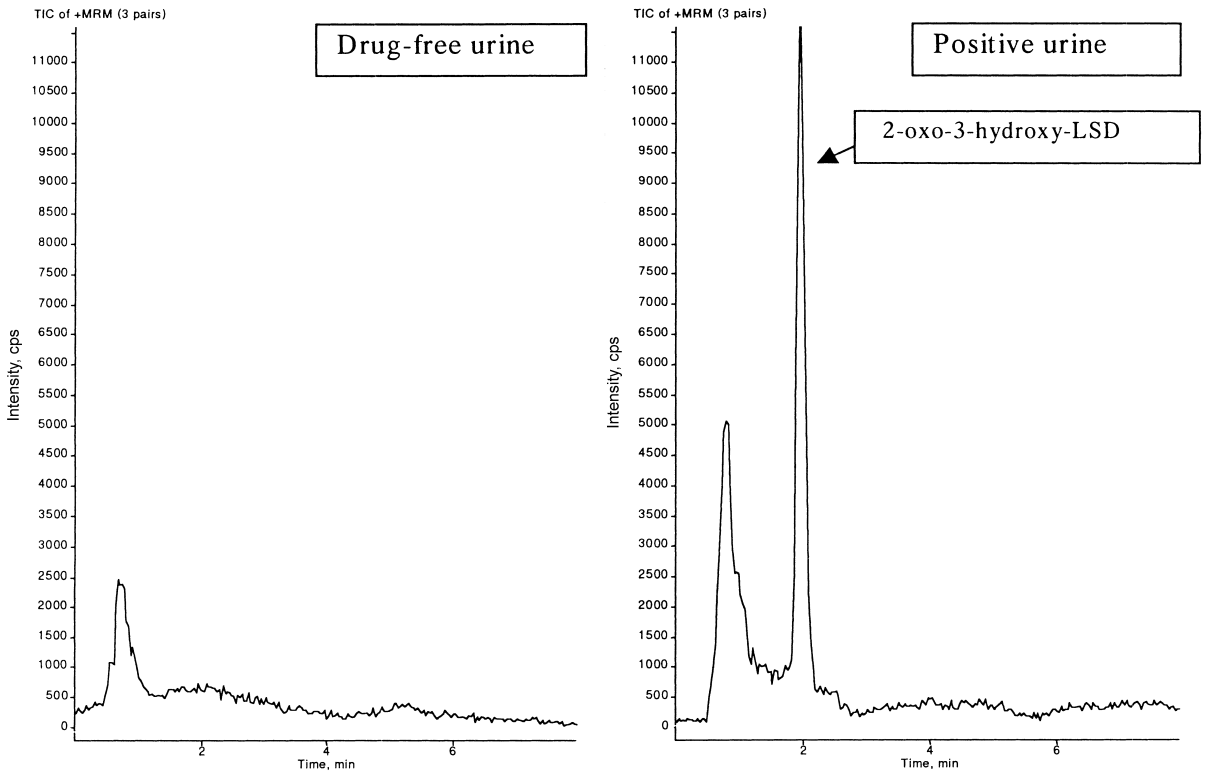

Fig. 6. O–H–LSD detection in a drug-free (left) and in a positive LSD urine sample.

μg/l for case 1 and 2, respectively) was done using deuterated LSD as internal standard. This metabolite was not found in plasma.

3.5.2. Identification of trioxylated-LSD (MH^+ = 372)

This compound with molecular mass of 371 (i.e. 48 higher than LSD) was described by Cai and Henion [2] in human liver microsomal incubation. Using 372/253 (loss of diethylamide and H_2O) and 372/238 (diethylamine, $CH_2{=}N{-}CH_3$ and H_2O) transitions, a possible trioxylated-LSD metabolite was detected at a retention time of 0.9 min (Fig. 7). At our knowledge, this compound has not been described in human urine.

3.5.3. Identification of lysergic acid ethyl-2-hydroxyethylamide (LEO) (MH^+ =340)

The position of hydroxyl group on branched side chain gives by MS–MS analysis, specific fragmentations. An ion chromatogram of MS–MS transition (340/223) corresponding to a neutral loss of an oxygen and diethylamide is shown in Fig. 8. The peak at retention time of 2.7 min was supposed to be LEO.

3.5.4. Identification of 13 and 14-hydroxy-LSD (MH^+ =340)

In order to identify 13 and 14-hydroxy-LSD in LSD-positive urine, we have compared ion current

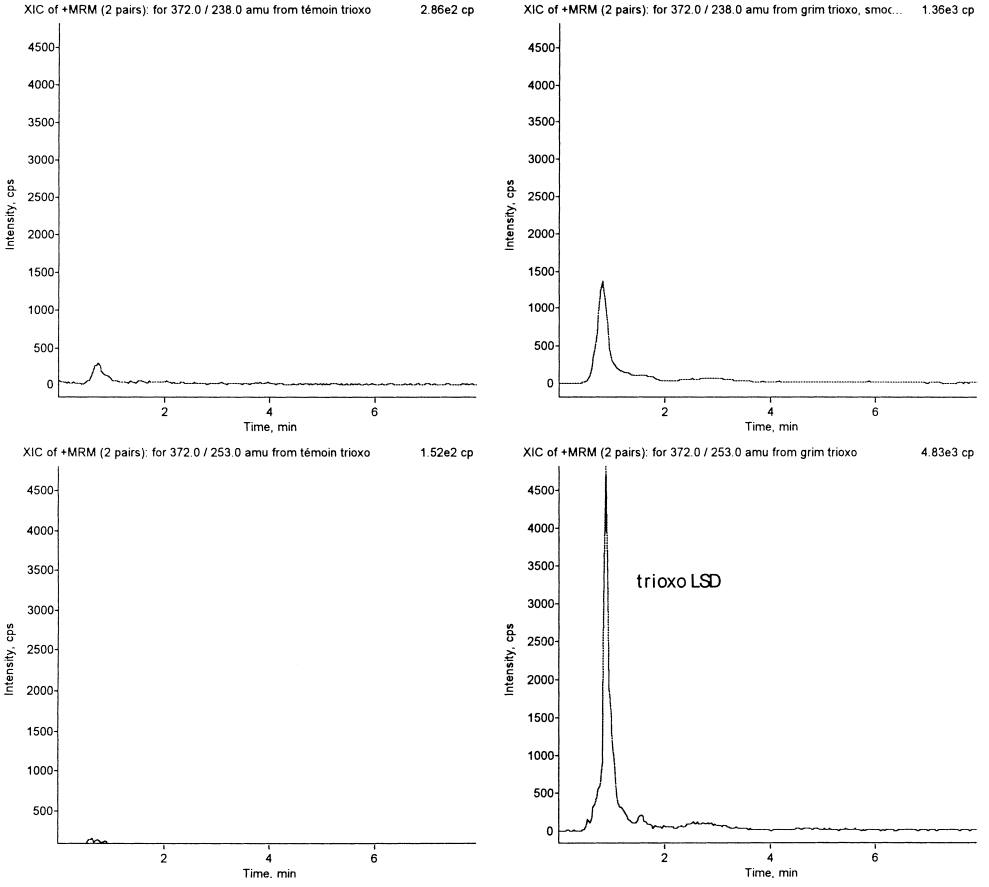

Fig. 7. Identification of trioxylated-LSD in drug-free (left) and in positive LSD urine sample on 372/238 (upper chromatogram) and 372/253 (lower chromatogram) MRM transitions.

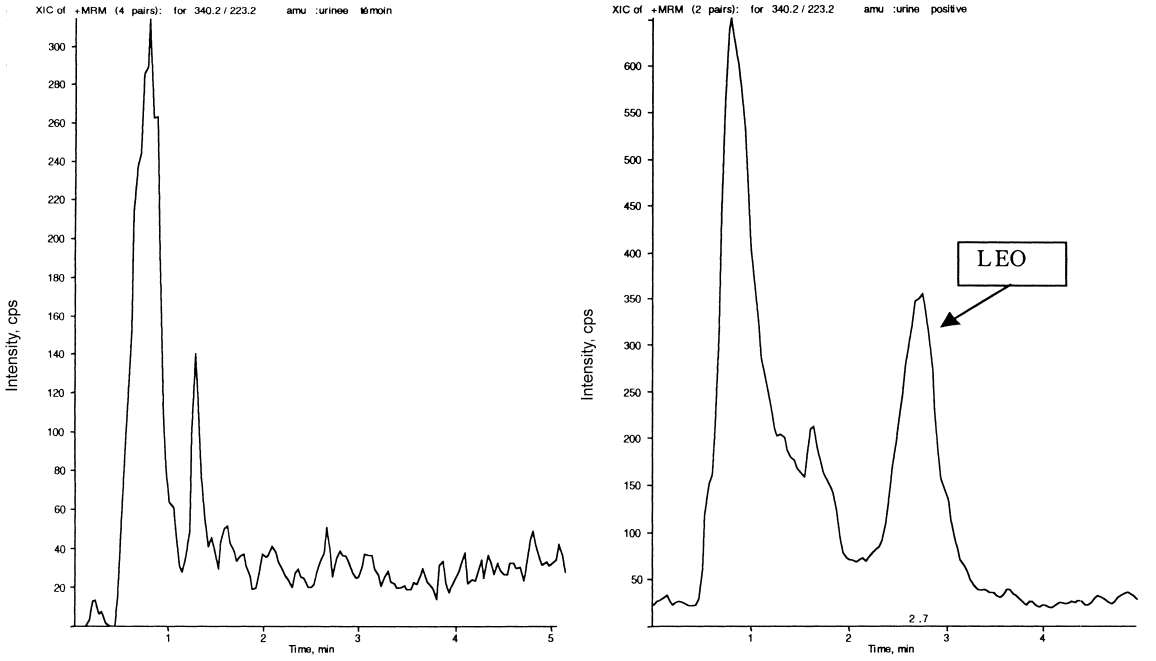

Fig. 8. Lysergic acid ethyl-2-hydroxyethylamide (LEO) determination in a drug-free and in a positive LSD urine sample.

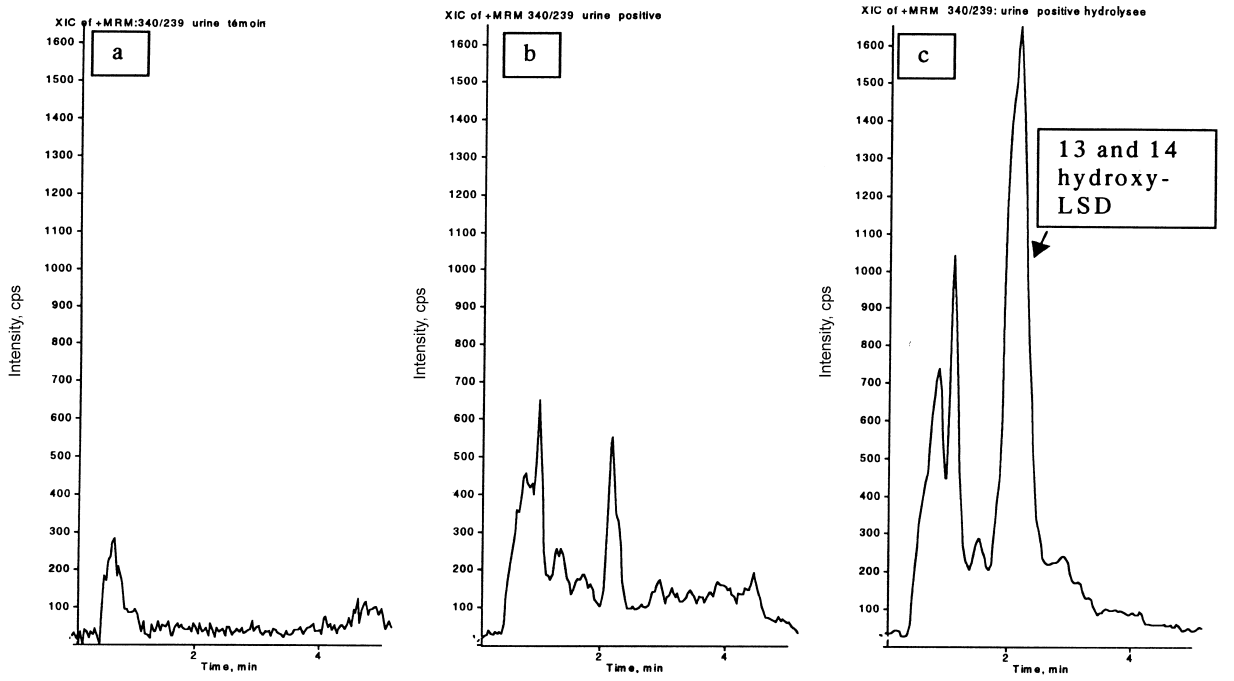

Fig. 9. 13 and 14-hydroxy-LSD determination (a) in a drug-free and in a positive LSD urine sample before (b) and after (c) after treatment with β-glucuronidase.

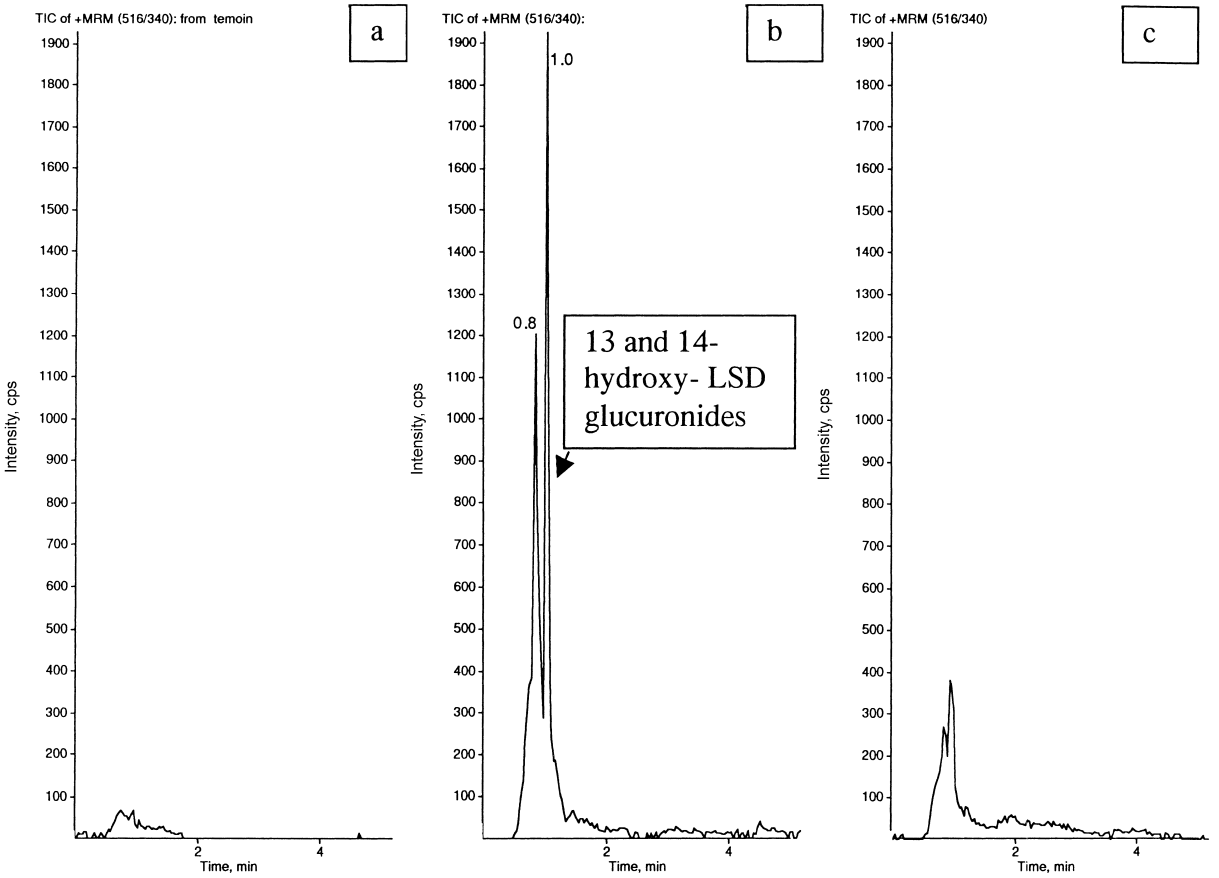

Fig. 10. 13 and 14-hydroxy-LSD-glucuronides determination in a drug-free (a), and positive urine sample before (b) and after hydrolysis (c) with β-glucuronidase.

profiles from LC–MS–MS analysis before and after treatment with β-glucuronidase. The ion chromatograms of MS–MS transition 340/239 (loss of diethylamide group) show that one peak at retention time of 2.3 min (Fig. 9) increases after hydrolysis. This peak probably contains coeluting 13 and 14-hydroxy-LSD. Identification of glucuronide conjugates of 13 and 14 hydroxy-LSD ($MH^+ = 516$) confirms the presence of hydroxy compounds. The selected MS–MS transition (516–340) corresponds to loss of glucuronic acid from the protonated molecule ($MH^+ - 176$). Corresponding chromatographic peaks appear at retention times of 0.85 and 1 min (Fig. 10). When the urine was incubated with β-glucuronidase before analysis, the two conjugated metabolite peaks drastically decreased.

4. Conclusion

Reliable monitoring of drugs of abuse requires a specific and sensitive analytical procedure. This is particularly true for LSD determination because this compound is active at very low levels. The LC–ESI–MS–MS method reported here, meets all of this criteria and detects easily quantitation levels as low as 0.02 μg/l for LSD in blood and urine.

The presence of potential metabolites can be observed using specific neutral loss scanning. In positive human urine we have detected the presence of several metabolites obtained after *N*-dealkylation: nor-LSD, nor-iso-LSD and lysergic acid ethylamide or after oxidation and hydroxylation: 2-oxo-3-hydroxy-LSD, trioxylated-LSD, lysergic acid ethyl-2-

hydroxyethylamide, 13 and 14-hydroxy-LSD and their glucuronide conjugates. At our knowledge trioxylated-LSD and lysergic acid ethyl-2-hydroxy-ethylamide were never described in human urine. The main metabolite O–H–LSD was not found in plasma but in urine this compound was two times higher in case 1 and 25 times higher in case 2 than the parent drug. Detection of this metabolite in urine can increase the period for confirming LSD ingestion.

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