Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 825 (2005) 21–28
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatography–tandem mass spectrometry determination of LSD, ISO-LSD, and the main metabolite 2-oxo-3-hydroxy-LSD in forensic samples and application in a forensic case

Sys Stybe Johansen*, Jytte Lundsby Jensen

Department of Forensic Chemistry, Institute of Forensic Medicine, University of Copenhagen, Frederik V's vej 11, DK-2100 Copenhagen Ø, Denmark

Received 16 October 2004; accepted 22 December 2004
Available online 26 January 2005

Abstract

A liquid chromatography mass spectrometric (LC/MS/MS) method has been developed for the determination of LSD, iso-LSD and the metabolite 2-oxo-3-hydroxy-LSD in forensic applications. The procedure involves liquid–liquid extraction of the analytes and LSD-D_3 (internal standard) from 1.0 g whole blood or 1.0 ml urine with butyl acetate at pH 9.8. Confirmation and quantification were done by positive electrospray ionisation with a triple quadrupole mass spectrometer operating in multiple reaction monitoring (MRM) mode. Two MRM transitions of each compound were established and identification criteria were set up based on the retention time and the ion ratio. The curves of extracted standards were linear over a working range of 0.01–50 μg/kg for all transitions of LSD and iso-LSD. The limit of quantification was 0.01 μg/kg for LSD and iso-LSD. The method was applied to a case investigation involving a 26-year-old male suspected for having attempted homicide, where blood concentrations of LSD and iso-LSD were determined to 0.27 and 0.44 μg/kg, respectively. 2-Oxo-3-hydroxy-LSD was detected in the urine and confirmed the LSD abuse. The case illustrated the importance of analyte separation before MRM detection of a sample due to identical fragmentation ions of the isomers.

Keywords: LSD; ISO-LSD; Whole blood; LC/MS/MS

1. Introduction

Lysergic acid diethylamide (LSD) illustrated in Fig. 1 is a very potent hallucinogenic substance. It is considered a highly dangerous drug due to its tendency to produce panic, delirium and bizarre behaviour, sometimes resulting in irrational and injurious acts [1–4]. LSD acts on multiple sites at the CNS. Its best studied effects are the agonistic actions at presynaptic receptors for 5-hydroxy-tryptamine in the midbrain [1,5]. LSD is an illegal drug commonly referred to as "acid" and it does not occur in nature but is easily prepared from ergotamine and related ergot alkaloids [6]. LSD is colourless, odourless and tasteless and primarily found on blotter paper [1–5]. The LSD consumption has increased since the beginning of the 1990s after a drop in the 1980s. The active oral dose is about 20–100 μg, but sometimes up to 500 μg is used [1–4,7]. The active blood level concentration of LSD is very low (maximum plasma concentrations following 70 μg dose range up to 2 μg/kg). The plasma elimination half-life of LSD has been reported to be 3–6 h [1–5]. LSD is extensively metabolized in the liver and less than 1% of the drug is eliminated unchanged in the urine [1–4]. Because of the low blood concentrations, LSD analysis is often performed on urine. Nor-LSD, 2-oxo-3-

Abbreviations: BIAS, systematic errors, i.e. proximity to the true value; ESI, electrospray-ionisation; GC/MS, gas chromatography mass spectrometer; IS, internal standard; Iso-LSD, iso-lysergic acid diethylamide (isomer); LC/MS/MS, liquid chromatography mass spectrometer (tandem); LOQ, limit of quantification; LSD, lysergic acid diethylamide; LSD-D_3, deuterated LSD (internal standard); M-LSD, 2-oxo-3-hydroxy-LSD; MRM, multiple reaction monitoring; MRM1, MRM transition 1; *N*, number of samples; R^2, correlation coefficient; RIA, radioimmunoassay; R.S.D., relative standard deviation

* Corresponding author. Tel.: +45 35326241; fax: +45 35326085.
E-mail address: ssj@forensic.ku.dk (S.S. Johansen).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.12.040

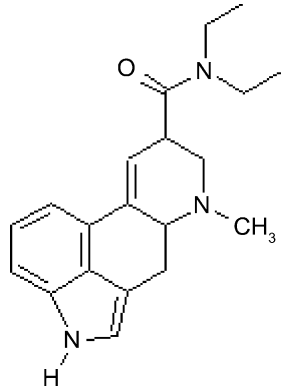

Fig. 1. Chemical structure of LSD.

hydroxy-LSD and the glucuronide conjugate of 13-hydroxy-LSD can be detected in urine up to 96 h after administration, whereas LSD can only be detected for 12–24 h post administration. 2-Oxo-3-hydroxy-LSD is present in human urine at concentrations 16–43 times greater than LSD, the parent compound [8]. Iso-LSD (an inactive isomer at C-8) is often found in urine, but it is a contaminant of LSD itself.

As a result of the high potency and low doses, detection of LSD in biological matrices poses a significant analytical challenge. Direct GC/MS is of limited use because of the irreversible adsorption of LSD on GC columns and because of its low volatility and thermal instability at GC temperatures. Selective extraction and derivatization by silylation of the indole nitrogen of LSD is necessary when using GC/MS. Alternatively HPLC separation together with fluorescence detection has been applied, however, it lacks sensitivity and specificity [7–12]. Consequently, LC/MS/MS is studied as the method of choice for LSD determination in biological samples.

Most publications describe methods for urine due to the high sensitivity demands [10–12]. Only few papers have described analytical methods sensitive enough for determination of LSD and related analytes in blood [13,14]. Faverretto et al. [13] described a simple LC/MS/MS using step liquid–liquid extraction of biological samples with the carcinogenic solvent chloroform, while Canezin et al. [14] describe a LC/MS/MS method for plasma and urine with a similar one step liquid–liquid extraction using diethylether (extremely explosive). Both methods involved hazardous solvents but described sufficient sensitivity with acceptable sample size. Other more complicated methods involving difficult and time-consuming sample preparation are described such as a LC/MS method by Bodin and Svensson [15] involving extraction into an organic solvent (diisopropylether) and back-extraction. Sklerov et al. [16] reported a LC/MS method for whole blood of a combined liquid–liquid extraction and solid-phase extraction. Many earlier methods lack the required sensitivity for measuring LSD in blood such as Bogusz et al. [17], who used LC–APCI–MS for routine in multipleanalyte analysis. Use of tandem mass spectrometry clearly improves and simplify the sample preparation.

The Department of Forensic Chemistry at the Institute of Forensic Medicine performs the toxicological analysis on the driving and criminal cases (offences) in Denmark and the post-mortem cases of East Denmark requested by the police. This paper describes a LC/MS/MS method using positive ESI with a triple quadrupole mass spectrometer for the determination of LSD and related analytes in biological samples from forensic cases. A case is studied involving a 26-year-old male suspected of having attempted homicide. The method describes a new simple sample preparation method of forensic samples involving one-step liquid–liquid extraction using a more environmental safe/less hazardous solvent than earlier described methods.

2. Experimental

2.1. Chemicals and materials

LSD was acquired from Lipomed GmbH (Germany), iso-LSD and the internal standard (IS) LSD-D_3 and 2-oxo-3-hydroxy-LSD (M-LSD) from Promochem Standard Supplies AB (Sweden)—all of high purity. Solvents of chromatographic grade from Rathburn Chemicals Ltd. (UK) and Merck (Germany) were used for extraction and analysis. Sodium carbonate was of analytical grade from Merck (Germany). Control whole blood was obtained from horses, while urine samples were achieved from healthy volentriers in the laboratory.

2.2. Stock solutions and standards

Standards from 0.01 to 50 μg/l with 5.0 μg/l IS were prepared in mobile phase, while extracted standards were made from 0.01 to 50 μg/kg whole blood or 0.01 to 50 μg/l urine as described below. Blood samples were weighed prior to analysis as a standard procedure in the laboratory with whole blood and post-mortem blood, while urine samples were dispensed.

2.3. Sample preparation (protected from light)

A 1.0 g whole blood or 1.0 ml urine was mixed with 20 μl 0.05 mg/l aqueous LSD-D_3. The sample was extracted with 1.0 ml butyl acetate after adjusting pH with 1.0 ml 1 M sodium carbonate. After centrifugation for 10 min at 3600 rpm, the organic fraction was removed and evaporated to dryness at 40 °C under a steam of pure nitrogen. It was reconstituted in 100 μl mobile phase and transferred to a brown autosampler vial and 20 μl was injected into the chromatographic system.

Table 1
MS/MS conditions for the Quattro Micro

	Collision energy 1 (V)	Collision energy 2 (V)	Transition 1, MRM1	Transition 2, MRM2	Ion ratio, MRM2/MRM1
LSD	25	30	324 → 223	324 → 208	0.61
Iso-LSD	21	30	324 → 281[a]	324 → 208	1.0
M-LSD	23	30	356 → 237	356 → 222	0.84
IS (D_3-LSD)	25	–	327 → 226	–	–

M-LSD: 2-oxo-3-hydroxy-LSD.
[a] Iso-LSD also has the transition 324 → 223.

2.4. Chromatography and MS/MS conditions

For separation an Agilent 1100 series HPLC system consisting of binary pump, autosampler, and thermostatted column compartment was used with a Zorbax SB-C18 column (2.1 mm × 30 mm, 3.5 μm). Gradient elution began with a mobile phase of 5% of solvent A (5% acetonitrile with 0.05% formic acid) and 95% of solvent B (100% acetronitrile with 0.05% formic acid). This composition was held for 2 min and then the percentage of solvent B was linearly increased to 60% over 10 min. A total analysis time of 20 min was used due to the re-equilibration time between injections. The flow rate was 0.2 ml/min at 30 °C.

A Quattro Micro, tandem quadrupole mass spectrometer, Waters, was coupled to the HPLC system. Data was acquired in the positive ion mode with an electrospray (ESI) source using MassLynx software 4.0, and calculations used extracted ion chromatograms by QuanLynx. Mass spectrometer conditions (cone, lens voltage, collision energy, etc.) were optimised by direct infusion of the standard into the Z-spray ion source. Multiple reaction monitoring (MRM) analysis was used for data collection (see Table 1). The cone energy was optimised at 30 V, while the source and desolvation temperatures were 120 and 350 °C, respectively.

An ion suppression experiment was performed by injecting LSD and iso-LSD through the syringe pump into a T-piece with mobile phase. Suppression was examined in an extracts of control whole blood within a normal run time.

3. Results and discussion

3.1. Chromatography and MS/MS conditions

The presence of metabolites, inactive isomers, etc. with the same molecular masses and common fragments made the good chromatographic separation a necessity. A gradient system was setup that elute the compounds from 2 to 9 min: the internal standard and LSD eluted at the retention time of 7.60 min, M-LSD at 2.60 min, and iso-LSD at 8.80 min. Baseline separation was obtained between the analytes.

Screening of drug-free whole blood showed no endogenous interference at the retention times of the compounds and no ion suppression was observed on LSD or iso-LSD during a run after injection of whole blood extracts from control blood. However, the deutereted internal standard also illustrates (by change in recovery) and corrects any possible suppression of the samples.

As identification criteria both relative and absolute retention times can be used. The absolute retention time may have a maximum deviation of ±0.1 min or the relative time when using the internal standard may have a maximum deviation of ±2%. For each compound of interest two MRM's were set up, one for quantification and one as "qualifier" using one parent ion and two daughter ions per compound as shown in Table 1. Furthermore, the ratio of the responses for MRM2 against MRM1 may have a maximum deviation of 10%. By using this principle we fulfil the requirements in EU guidelines on environmental and food analyses—this method give four identification points according to EU guidelines/SANCO rules [18], where three or more are requested as a minimum. Finally, concerning the identification criteria, detection of the metabolite, 2-oxo-3-hydroxy-LSD confirmed the intake of LSD [8]. The urine data are given although the sample preparation and MS detection is not optimized for M-LSD. M-LSD is used as a marker (qualitative) for LSD abuse and an alternative detection that support the blood data.

The recovery of internal standard LSD-D_3 from extracted whole blood standards was 65% (absolute) compared to pure standards and similar extraction efficiencies were obtained for LSD, iso-LSD and M-LSD. This is a high and acceptable recovery because a loss occurs when the organic phase is transferred. The recovery is comparable with other substances such as loperamide and methadone [19,20].

3.2. Linearity and limit of quantification

A linear correlation was observed from 0.02 to 200 μg/l in pure standards made up in mobile phase with acceptable correlation coefficients and residuals. The linear function of LSD in pure standards was $174x - 0.0007$ with a correlation coefficient (R^2) of 0.9997 for MRM1 and $115x - 0.0002$ with R^2 of 0.9996 for MRM2.

The calibration curves of extracted whole blood standards were linear over a working range of 0.01–50 μg/kg for all four transitions of LSD and iso-LSD. The calibration graphs were derived by plotting the peak area ratio of analyte to the IS versus the whole blood concentration of the analyte: linear regression with $1/x$ weighting was used. The

Table 2
Linear parameters (range, function, correlation coefficient) and limit of quantification (LOQ)

		LSD MRM1	Iso-LSD MRM1	M-LSD[a] MRM1
Blood	Linear range (μg/kg)	0.01–50	0.01–50	
	Function	$F(x) = 1750x + 0.0044$	$F(x) = 1610x - 0.0014$	
	R^2	0.9994	0.9984	
	LOQ (μg/kg)	0.01	0.01	
Urine[b]	Linear range (μg/l)	0.01–50	0.01–50	0.5–50
	Function	$F(x) = 1680x + 0.0009$	$F(x) = 1610x - 0.0140$	$F(x) = 44.2x + 0.002$
	R^2	0.9978	0.9984	0.9926
	LOQ (μg/l)	0.01	0.01	0.5

[a] Not optimised and validated only for qualitative work.
[b] Urine samples are mostly used for qualitative work and as an alternative matrix.

linear function of LSD and iso-LSD for MRM1 are given in Table 2. All had acceptable residuals. In Fig. 2, typical standard curves of LSD are shown. The linear function of M-LSD in urine are shown in Table 2. The linear range was assessed from 0.5 to 50 μg/l, however, urine samples are mostly used for qualitative work and therefore only LSD and iso-LSD were optimised and validated for quantitative analysis in this study.

The limit of quantification was 0.01 μg/kg for LSD and iso-LSD in whole blood as illustrated in Fig. 3. The limit of quantification was defined as the lowest concentration at which the signal-to-noise level (S/N) of the extracted sample was higher than 10. All four transitions fulfilled this S/N level. The sensitivity of transition 2 was a little better than transition 1 of both compounds as shown in Fig. 3.

3.3. Precision and accuracy

The intra-day repeatability expressed by relative standard deviation (R.S.D.) studied at several concentration levels in whole blood was generally less than 10% for both analytes as illustrated in Table 3. The R.S.D. was highest at limit of quantification. The inaccuracy did not exceed 14% for LSD and iso-LSD, and the accuracy was better for LSD than iso-LSD probably due to the nature of the internal standard.

3.4. Case investigation

The method was applied for a case investigation involving a 26-year-old male in connection with attempted homicide. He admitted to have been abusing LSD and the analysis was

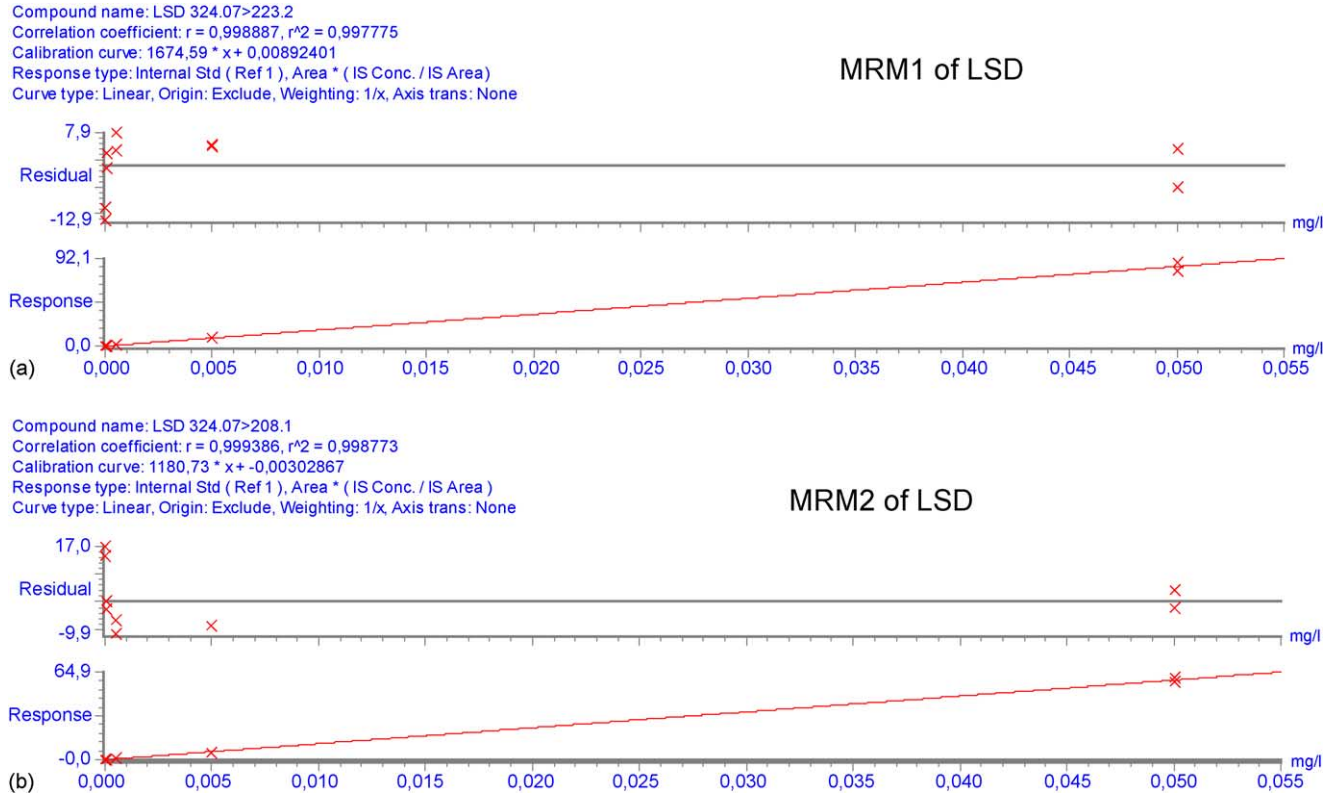

Fig. 2. Standard curves of extracted whole blood with LSD of transitions 1 and 2 including plot of the residuals.

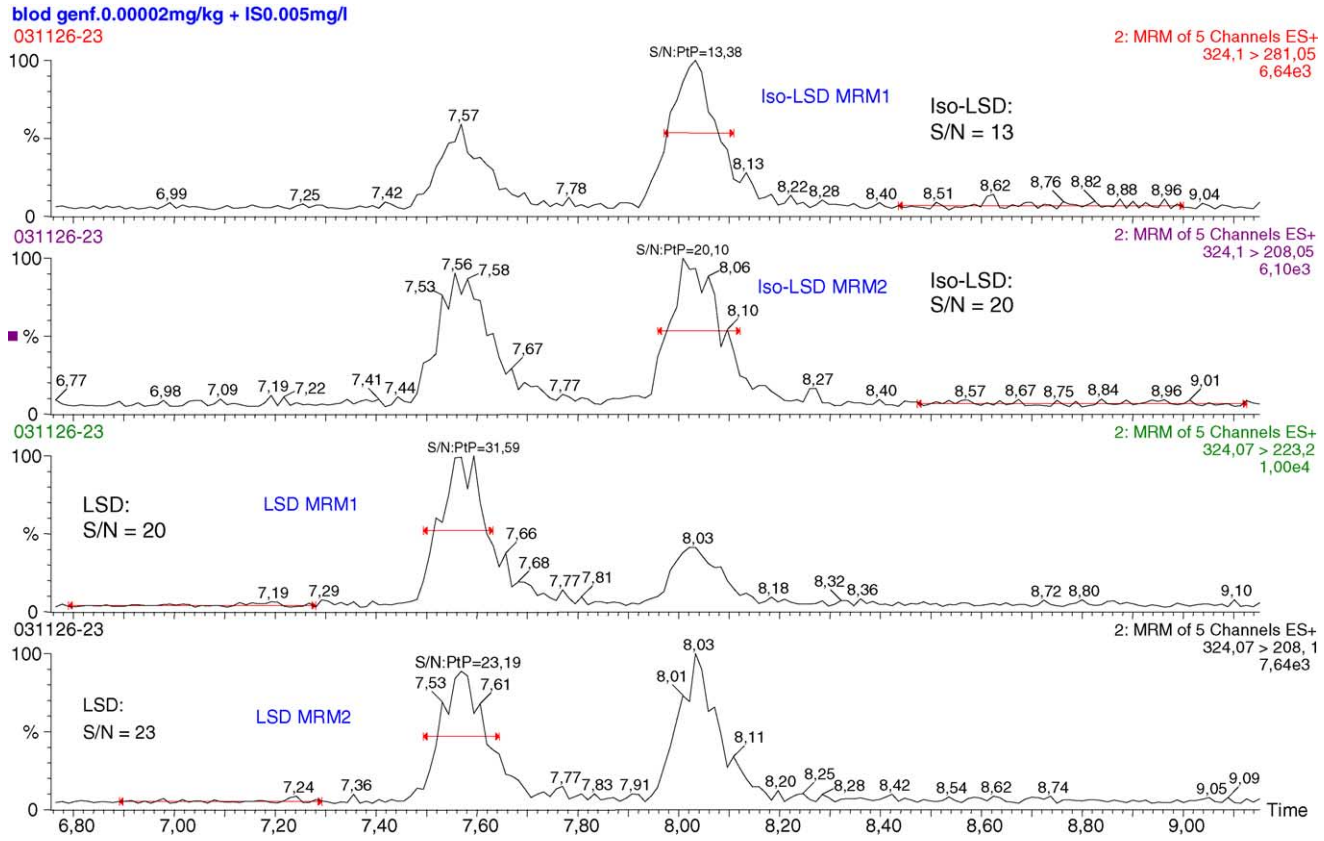

Fig. 3. LC/MS/MS chromatograms of spiked whole blood at 0.02 μg/kg of LSD at 7.6 min and iso-LSD at 8.0 min, respectively. The absolute recovery of the internal standard was 65%. For each compound their two MRM transitions are shown with the signal to noise level (S/N).

therefore requested. The described LC/MS/MS method was applied, and blood concentrations of LSD and iso-LSD were determined at 0.27 and 0.44 μg/kg, respectively (see also Table 4). The identification criteria were fulfilled concerning the retention time and ion ratio. The internal standard was recovered about 85% in the whole blood sample—relatively. A chromatogram of the whole blood extract is shown in Fig. 4 together with a blank blood extract.

Illegally produced LSD contains often the impurity iso-LSD—an inactive diastereoisomer that is formed during the synthesis from lysergic acid. This isomer was detected in the whole blood (see Table 4 and Fig. 4).

Table 3
Precision and accuracy results based on studies made on several days (intra-laboratory)

	Concentration added (μg/kg)	Mean (μg/kg)	BIAS %	R.S.D. %	*N*
LSD	0.02	0.022	11.2	22.8	8
	0.05	0.050	−0.2	6.6	12
	0.50	0.509	1.9	8.1	8
	5.00	5.29	5.9	9.1	8
Iso-LSD	0.02	0.022	11.3	7.7	4
	0.05	0.053	6.0	3.4	4
	0.50	0.434	−13.5	3.3	4

N: number of samples.

The metabolite, 2-oxo-3-hydroxy-LSD, was detected at about 8.8 μg/l in the urine sample and confirmed the abuse of LSD. Free LSD and iso-LSD were also detected in the urine sample at 3.0 and 2.4 μg/l although only 1–4% of oral dose excreted in the urine as unchanged LSD see Fig. 5.

It has been demonstrated that M-LSD is present in human urine at concentrations 16–43 times greater than LSD, the parent compound. This study observed a ratio of about 3. Notice that it is only free LSD that is determined. Canezin et al. [14] reported a case of similar levels. The presence of M-LSD is a direct result of LSD metabolism in human system [8] conforming the blood results. Furthermore, the detection of the metabolite in urine can increase the period for confirmation of LSD ingestion. This is very relevant because of the short half-life of LSD [1].

The case illustrated the importance of analyte separation before selective monitoring reaction mode detection of a sam-

Table 4
The measured concentrations of LSD and related compounds in blood and urine from a 26-year-old male

	LSD	Iso-LSD	M-LSD
Whole blood (mg/kg)	0.27	0.44	N.D.
Urine[a] (mg/l)	3.0[a]	2.4[a]	8.8

N.D.: not detected.
[a] Free concentration.

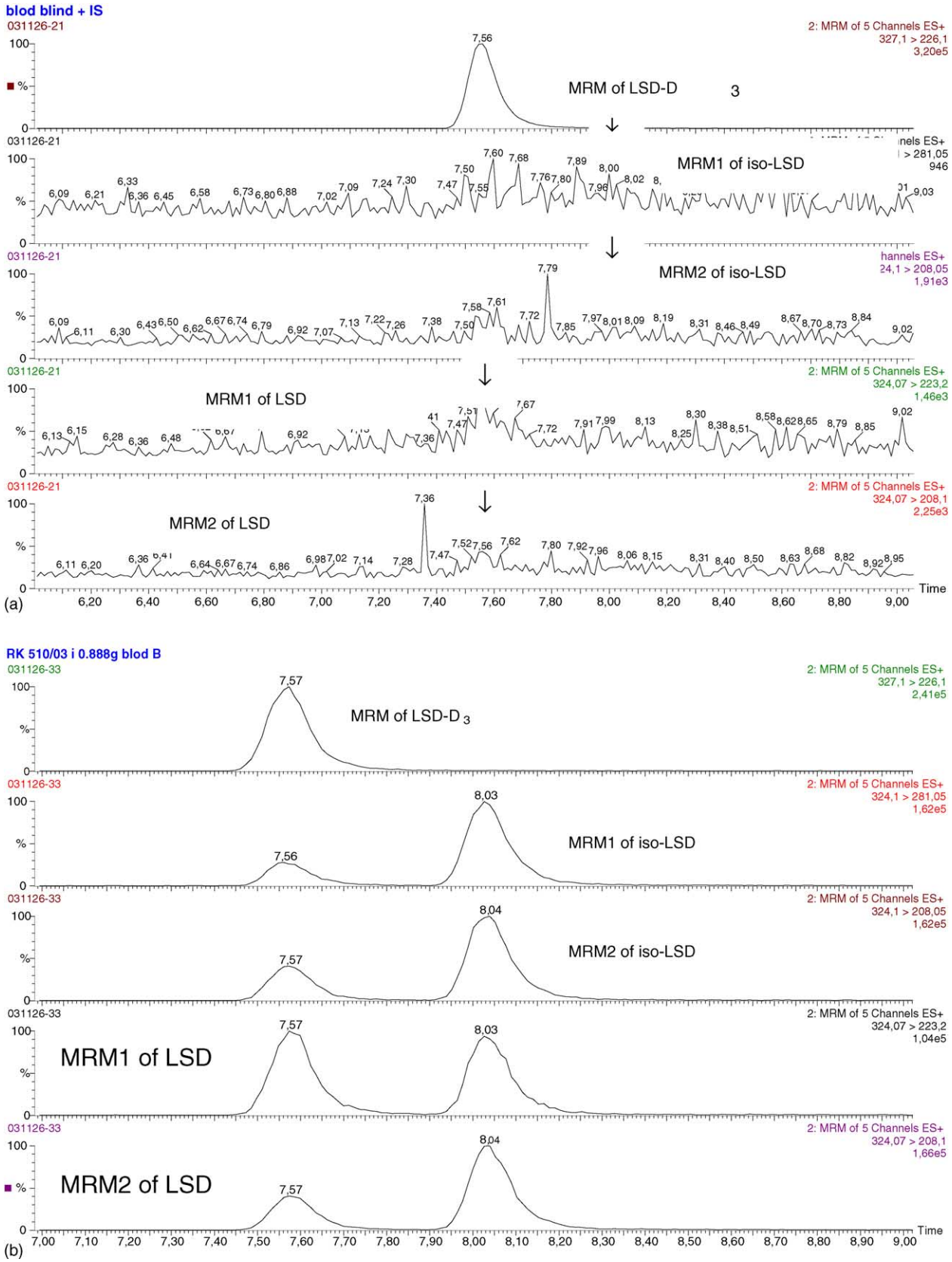

Fig. 4. (a) A typical LC/MS/MS chromatogram of blank whole blood with internal standard (recovery: 75%). S/N < LOD. The arrows indicate the retention time of LSD and iso-LSD. (b) A chromatogram of an extracted whole blood sample from a 26-year-old male who admitted having abused LSD. The relative recovery of the internal standard was 85% in his blood sample. All identification criteria were fulfilled for all analytes. The determined concentrations are listed in Table 3.

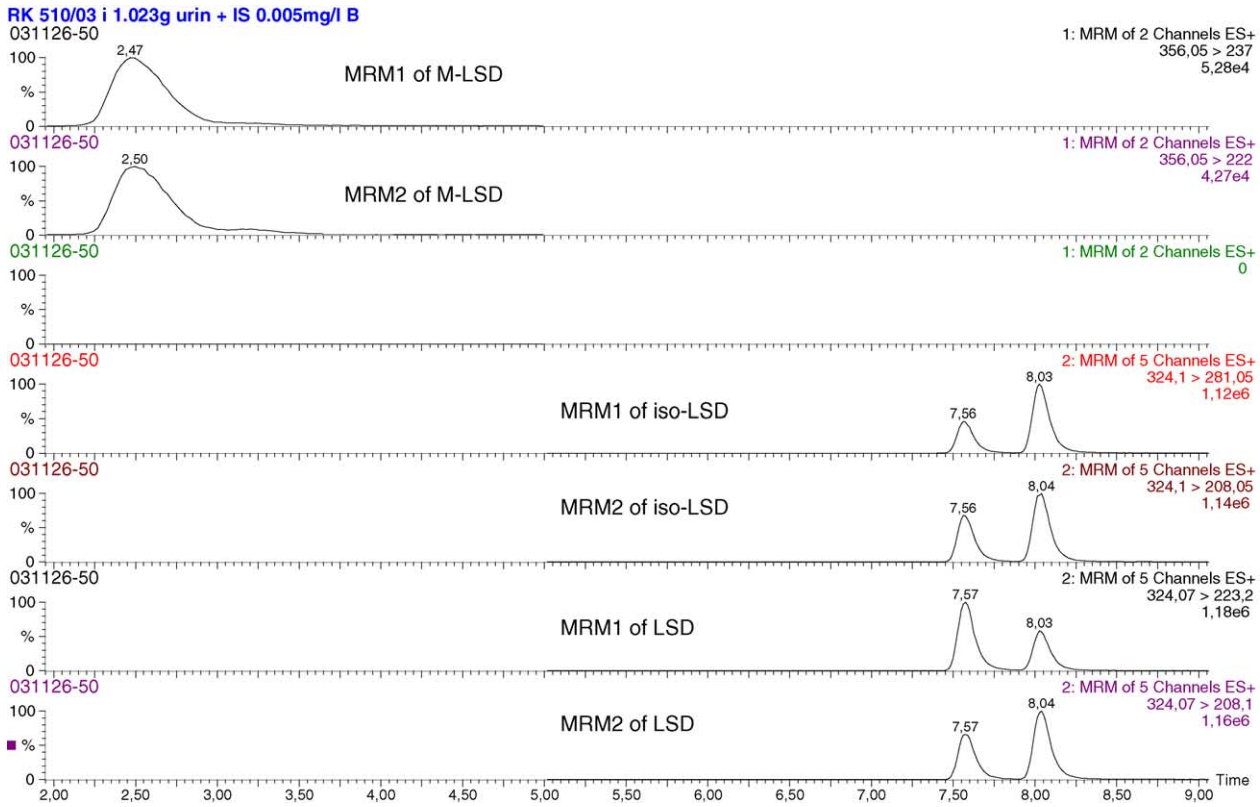

Fig. 5. A chromatogram of an extracted urine sample from the 26-year-old male. The relative recovery of internal standard was 91% in the sample. The positive sample contained 8.8 μg/l M-LSD, 3.0 mg/l free LSD and 2.4 μg/l free iso-LSD. All identification criteria were fulfilled.

ple. The chromatographic separation of LSD and iso-LSD were of importance because the diastereoisomers have identical fragmentation paths leading to fragments of equal mass to charge size.

4. Conclusions

A simple LC/MS/MS application has been set up to identify, confirm and quantify LSD and other related analytes in forensic samples. Before this simple LC/MS/MS method was developed radioimmunoassay (RIA) was used for screening of LSD and metabolites, but now RIA is stopped because it is too time-consuming and expensive considering the low number of cases per year. For instance the kit got to old between the cases and this increased the expedition time of the case (long delivery time of RIA kit). The LC/MS/MS method is used as a target analysis that identify, quantify and confirm. No alternative method is used instead the measurements are done in two matrices (blood and urine for verification) if possible.

The described method is simple and more environmental friendly than earlier published methods. The required sensitivity for measuring LSD and related analytes in blood are achieved. The LC/MS/MS method is definitely more convenient than GC/MS for confirmation of LSD abuse.

References

[1] O.H. Drummer (Ed.), The forensic Pharmacology of drugs of abuse, Arnold, London, 2001, p. 323, Chapter 7.
[2] S.J. Jenkins, in: B. Levine (Ed.), Principles of Forensic Toxicology, American Association for Clinical Chemistry, USA, 1999, p. 286, Chapter 16.
[3] J.T. Cody, in: M.J. Bogusz (Ed.), Forensic Science. Handbook of Analytical Separations, vol. 2, UK, 2000, p. 143.
[4] S.C. Sweatman (Ed.), Martindale. The Complete Drug Reference, London, PhP, 2002.
[5] A. Goodman Gilman, T.W. Rall, A.S. Nies, P. Taylor, The Pharmacological Basis of Therapeutics, Pergamon Press, USA, 1990, p. 554.
[6] C.H. Nelson, R.L. Foltz, J. Chromatogr. 580 (1992) 97.
[7] S. Schneider, P. Kuffer, R. Wennig, J. Chromatogr. B 713 (1998) 189.
[8] K.L. Klette, C.J. Anderson, G.K. Poch, A.C. Nimrod, M.A. Elsohly, J. Anal. Tox. 24 (2000) 550.
[9] J.F. Van Boclaer, K.M. Clauwaert, W.E. Lambert, D.L. Deforce, E.G. Van den Eeckhout, A.P. De Leenheer, Mass Spectrom. Rev. 19 (2000) 165.
[10] F.E. Dussy, C.H. Stäuble, T.A. Briellmann, Chimia 56 (2002) 53.
[11] P. Marquet, G. Lachatre, J. Chromatogr. B 733 (1999) 93.
[12] H.H. Mauer, J. Chromatogr. B. 713 (1998) 3.
[13] D. Faverotte, G. Frison, L. Maietti, L. Tedeschi, S.D. Ferrara, Ann. Toxicol. Anal. XIV 3 (2002) 201.
[14] J. Canezin, A. Cailleux, A. Turcant, A. Le Bouil, P. Harry, P. Allain, J. Chromatogr. B 756 (2001) 15.
[15] K. Bodin, J. Svensson, Ther. Drug Monit. 23 (2001) 389.

[16] J.H. Sklerov, J. Magluido Jr., K. Shannon, M.L. Smith, J. Anal. Tox. 24 (2000) 543.
[17] M.J. Bogusz, R.D. Maier, K.D. Krüger, U. Kohls, J. Anal. Tox. 22 (1998) 549.
[18] SANCO, Off. J. Eur. Commun. 221 (2002) 8.
[19] S.S. Johansen, J.L. Jensen, J. Chromatogr. B 811 (2004) 31.
[20] S.S. Johansen, Quantification of methadone in forensic samples by LC/MS/MS, in: Proceedings of the 39th International Association of Forensic Toxicologists Meeting, Prague, Czech Republic, 2001, p. 412.