ELSEVIER

Journal of Chromatography B, 713 (1998) 189–200

JOURNAL OF CHROMATOGRAPHY B

Review

Determination of lysergide (LSD) and phencyclidine in biosamples

S. Schneider*, P. Kuffer, R. Wennig

Laboratoire National de Santé, Centre Universitaire de Luxembourg 162A, avenue de la Faïencerie L-1511, Luxembourg, Luxembourg

Abstract

Lysergic acid diethylamide (LSD) is difficult to detect and to quantify in biosamples because of its very low active dose. Although there are a number of tests available, routine analysis of LSD is rarely performed. Immunoassays largely vary in their specificity and cross-reactivities with other molecules often make these tests unreliable. Because of its low concentration and the instability of the derivatives (e.g. trimethylsilyl-LSD), routine gas chromatography–mass spectrometry (GC–MS) detection and quantitation of LSD remains a difficult task. The most promising procedures for LSD determination seems to be liquid chromatography–MS analysis using electrospray ionisation and selected ion monitoring (SIM). Extraction, derivatization, GC or high-performance liquid chromatography conditions and the different detection modes will be summarised.

Phencyclidine (PCP) is an abused drug seldom found outside the United States. Well established detection and quantitation procedures include radioisotopic and nonradioisotopic immunoassays and GC–MS analysis using SIM mode with deuterated PCP as internal standard. Alternatively, GC with nitrogen–phosphorus detection or capillary electrophoresis has been used. Recent progress in PCP analysis will be summarised.

Keywords: Reviews; Lysergide; LSD; Phencyclidine

Contents

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(97)00538-0

1. Lysergide (LSD)

1.1. Introduction

Claviceps purpurea (ergot) is a small fungal parasite of grain and grass seed heads known since the early history of agriculture. Eating the ergot can be fatal to both humans and animals. *C. purpurea* thus is at the origin of terrible mass poisoning since the middle ages [1]. In 1938 Stoll and Hofmann synthesized lysergic acid diethylamide (LSD) from lysergic acid, a hydrolysis product of *C. purpurea* alkaloids [2]. On April 16th, 1943, Hofmann unintentionally absorbed a minute amount of LSD. He reported seeing ''an uninterrupted stream of fantastic images of extraordinary plasticity... accompanied by an intense kaleidoscopelike play of colours'' [3]. This experience, the first LSD trip, lasted 3 h.

Today, *d*-LSD (the only psychoactive isomer) is one of the most potent mind-altering chemicals known. In the 1950s–1960s, clinical experimentation with LSD was conducted by psychiatrists but sometimes dramatic and unpleasant reactions occurred. Since 1966 LSD is an illegal drug, commonly referred to as ''acid''. LSD is odourless and tasteless; it is often added to absorbent paper, such as blotter paper and divided into small squares, each square representing one dose. The active dose is as little as 20–80 μg but sometimes up to 500 μg are used. Plasma and urine concentrations are in the sub-ng/ml range. According to a survey on drug abuse, LSD consumption is increasing since the beginning of the 1990s after a drop in the 1980s [4]. LSD is not considered to be physiologically addictive, but tolerance develops quickly when used daily which may be dangerous given the unpredictable effects of the drug.

1.2. Biological effects and pharmacokinetics

After ingestion of LSD, the hallucinogenic effects start after 30–90 min. They last between 5–14 h. The exact mechanism of LSD action is still poorly understood; it is however established that LSD acts as a serotonin analogue. Depending on its concentration and on the presence of other molecules, LSD has agonistic or antagonistic effects on the serotonin receptor family [6,7]. The plasma elimination half-life of LSD has been reported to be 5.1 h. LSD is extensively metabolized in the liver and less than 1% of the drug is eliminated unchanged in the urine [8].

The LSD metabolites identified are N-demethyl-LSD (nor-LSD), 13-hydroxy-LSD, 14-hydroxy-LSD [9] as well as, lysergic acid ethylamide (LAE) and 2-oxo-LSD [10] (Fig. 1). Today, nor-LSD is the only confirmed in vivo human metabolite. Other metabolites have been suspected but not identified [11].

1.3. Stability

The effect of freezing on LSD stability in urine has been studied by Paul et al. [12]. No significant loss of LSD was observed after 45 days at −18°C. It has been shown however that LSD is sensitive to UV light and pH values below 4. Thermal (in)stability is still a matter of discussion. According to some sources it is stable at 100°C [13,14], others report decomposition at 37°C [15].

1.4. LSD analysis

1.4.1. LSD in biosamples

Nelson and Foltz reviewed early development of LSD analysis in 1992 [9].

LSD detection often is performed using immunoassays. Confirmation and quantitation of LSD in body fluids is generally performed using gas chromatography (GC), capillary electrophoresis (CE) or reversed-phase high-performance liquid chromatography (RP-HPLC) and coupling one of these techniques to an mass spectrometer (MS). MS analysis and quantitation is generally carried out using selected ion monitoring (SIM). In fact, HPLC and GC systems are complementary; RP-HPLC with fluorescence detection being recommended for quantitation [16] and capillary GC using fused-silica bonded phase being the recommended technique for identification of ergot alkaloids [17].

1.4.1.1. *Immunoassays*. Commercial tests for qualitative LSD immuno testing are available from several suppliers (DPC, Roche, Boehring–Syva for example). They include radioisotopic (RIA) and nonRIA technologies [14,18,19]. The advantages of the non-RIA immunoassays are the relative low costs and easy handling because no special sample preparation

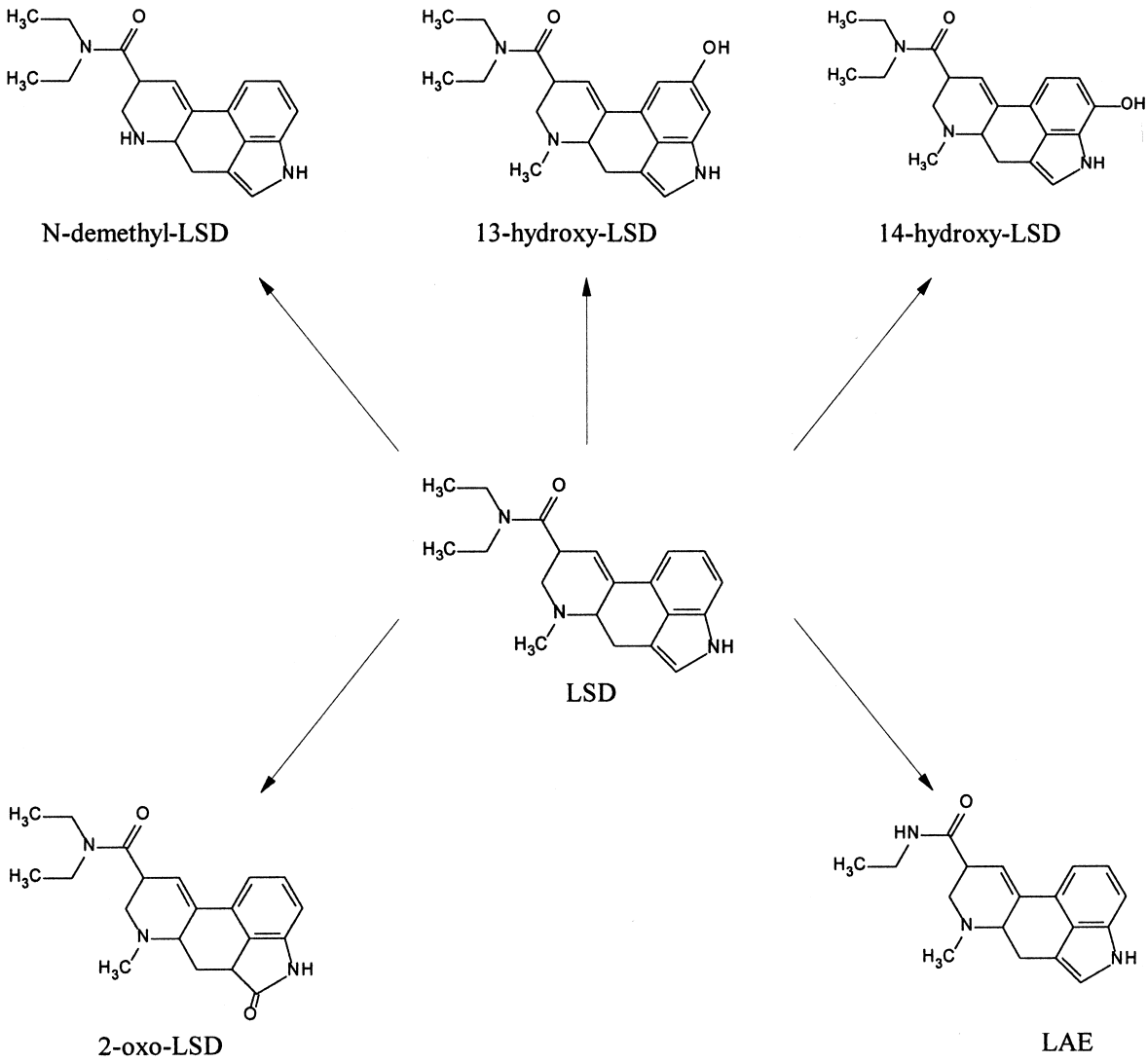

Fig. 1. Structures of LSD and its major metabolites.

is necessary. Costs of the RIA assays are high and handling and disposal of the radioactive material is difficult. RIA and the nonRIA assays generally have a cut-off value of 0.5 ng/ml in urine. High cross-reactivities were observed for nonRIA assays with nor-LSD and compounds such as neuroleptics and other drugs [20]. Recently, Cassells et al. [21] developed a solid-phase enzyme-linked immunosorbent assay immunoassay with specificity and sensitivity comparable to RIA assays. In general, however, positive immunoassay results always need confirmation by a second more specific method [19].

1.4.1.2. *Chromatographic analysis*. 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. The fragmentation pathway of

trimethylsilyl-LSD (TMS-LSD) and of LSD derivatives has been discussed by Paul et al. [13]. Retro-Diels reaction and cleavage of the deshydropiperidine moiety gives the fragments *m/z* 268, 253 and 279. Cleavage of the TMS from the LSD gives the peak at *m/z* 73. LSD fragmentation using electrospray ionisation (ESI) is discussed by Webb et al. [14]. The electron impact (EI) mass spectrum of TMS-LSD is represented in Fig. 2.

Since the late 1980s, ESI-MS has developed as an important technique for detection of molecules present at low concentrations such as LSD. The limitation of ESI-MS are the low liquid flow-rates and the need to avoid high aqueous content of the mobile phase. Because of its high cost and the need of highly trained manpower ESI-MS (as well as tandem MS), is still not used in many laboratories for routine analysis.

HPLC. HPLC separation together with fluorescence detection is a well-established method for analysis of trace amounts of LSD in biosamples. Methods based on HPLC separation and fluorescence detection have been reviewed by Nelson and Foltz [9]. Recently, Francis and Craston used affinity clean-up (ACU) for HPLC separation with fluorescence detection [22]. The mobile phase consisted of 35% aqueous ammonium acetate and 65% methanol. Optical excitation and emission were at 320 and 400 nm respectively. The limit of detection (LOD) was at 0.5 ng/ml of LSD in urine. The main advantage of this method is that large specimen volumes (up to 4 ml of urine when using 5 μg antibody) can be analysed in a relatively short time and that no derivatization step is necessary. The major drawback is that quantitation is not possible at high drug concentrations because of antibody saturation.

HPLC fluorescence was also used by Webb et al. for quantitative estimation of LSD concentration in urine before final confirmation by liquid chromatography (LC)–MS [14]. Separation was done using an ammonium acetate buffer at pH 8. The excitation wavelength was 330 nm and the emission wavelength was at 420 nm. The LOD was estimated to be 0.5 ng/ml. The linear range for quantitation was up to 10 ng/ml. Similar conditions were recently used by White et al. before ESI-MS confirmation [23]. The mobile phase of the HPLC was a mixture of 0.1 *M* acetate buffer–acetonitrile–triethylamine (75:25:0.25) at pH 8. Fig. 3 shows the separation of LSD and lysergic acid methylpropylamide (LAMPA) obtained under these conditions. Fluorimetric quantitation after HPLC separation was also used by Nakahara et al. [24]. The LOD was 0.05 ng/mg of human hair.

Musshof and Daldrup [5] described a sensitive method for LSD determination in serum samples. Liquid–liquid extraction (LLE) was performed using *n*-butyl chloride and derivatization using N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). GC–MS analysis in the SIM mode gave a linearity range for TMS-LSD from 0.1–10 ng/ml.

GC–MS. Nelson and Foltz developed a GC–MS–

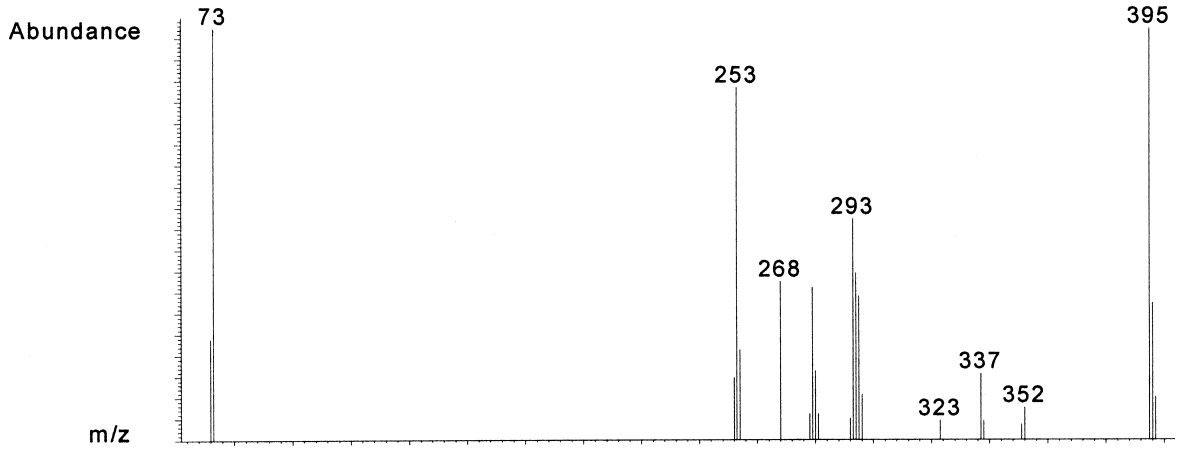

Fig. 2. EI mass spectrum of TMS-LSD.

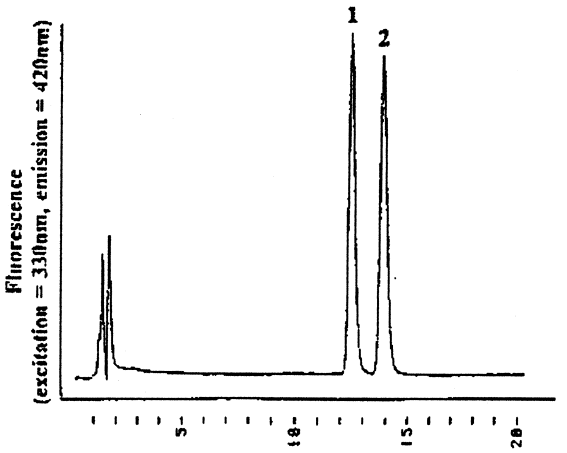

Fig. 3. HPLC separation of LSD (1) and LAMPA (2) [21].

MS method using either positive or negative chemical ionisation (CI) in order to detect LSD, iso-LSD and nor-LSD [25]. Ammonia and methane were used as reagent gases for the production of protonated (MH^+) and negative (M^-) ions. LOD for TMS-LSD and TMS-iso-LSD were found to be 10 pg/ml in spiked urine samples when using the positive ion CI; the calibration curves were linear from 20–1000 pg/ml. The LOD and limit of quantitation (LOQ) for nor-LSD were 100 pg/ml and 400 pg/ml, respectively. Detection and quantitation of iso-nor-LSD-TFA was possible down to 10 pg/ml and 50 pg/ml respectively. The same technique has been used by McNally et al. for confirmation of positive RIA results [18].

A recent publication discussed the GC–MS analysis of LSD and metabolite in rat and human hair after derivatization [24]. Using the SIM mode, LSD was detectable in rat pigmented hair following the lowest dose of intraperitoneal administration of LSD (0.05 mg/kg b. w.) and nor-LSD was detectable after the highest dose (2 mg/kg). In 2 out of 17 human hair samples of self-reported LSD users GC–MS analysis detected the presence of LSD, whereas nor-LSD was not detected. The internal standards used were d_{10}-LSD or LAMPA.

LC–MS and CE–MS. Rule and Henion proposed an ACU–HPLC–MS method to detect LSD in urine [26]. Extraction of the drug was performed by a high-performance protein G column primed with antiLSD antiserum to capture the LSD from urine. The ACU column was then coupled to a LC–MS system using an ion spray source and working in the SIM mode. The m/z 324 (MH^+) ion was used for SIM monitoring. The LOD was found to be 0.5 ng/ml.

High-flow electrospray LC–MS for detection of LSD was first introduced by Hopfgartner et al. [27]. The protonated form of LSD was analysed by HPLC using a 80:20 mixture of acetonitrile–water containing 5 m*M* ammonium acetate at a flow-rate of 1.2 ml/min. Using a special shield between the sprayer and the ion sampling capillary of the MS, they avoided column split and were able to use relatively high flow-rates. The LOD for LSD was 250 pg injected on-column with a dynamic linear range of two orders of magnitude.

Webb et al. proposed a LC–MS method for confirmation of positive immunoassays [14]. Again, affinity chromatography was used for isolation and clean-up of LSD. Conventional HPLC fluorescence allowed an estimation of the LSD concentration. Final confirmation and quantitation without derivatization were performed using LC–MS with ESI. By applying 10–20 V to an octapole rod assembly between the ESI source and the quadrupole analyser a characteristic fragmentation pattern for LSD was obtained with significant ions at m/z 223 and 281. The internal standard used in this study was methysergide. The LOD was found to be 0.5 ng/ml.

White et al. developed a LC–MS method for LSD analysis in urine, which has the advantage of avoiding derivatization [23]. The HPLC column was coupled to a mass spectrometer fitted with an electrospray interface. Using positive ion MS and monitoring the [MH^+] ion at m/z 324, the linear range for quantitation was 0.5–10 ng/ml. The internal standard used was methysergide.

Another LC–MS method using atmospheric pressure ESI was developed by Hoja et al. [28]. Clean-up of the sample was achieved using solid-phase extraction (SPE). HPLC was used for chromatographic separation using a mixture of ammonium formate buffer at pH 3 and acetonitrile (70:30). The MS parameters were optimised for the protonated molecular ion of LSD. LOQ were found to be 0.05 ng/ml and 0.10 ng/ml for LSD and nor-LSD respectively. The linear range of quantitation was 0.05–20 ng/ml.

Cai and Henion detected LSD and several of its

metabolites in liver microsomes by HPLC and CE coupled with ion-spray MS. They also discussed the fragmentation of LSD and of several analogues and identified LAE and 2-oxo-LSD [10]. CE separation was performed under a potential difference of 25.5 kV using a 100 cm×50 μm I.D. column. The CE electrolyte was ammonium acetate at pH 4.5 containing 20% methanol. The RP-HPLC was used with a linear gradient of two solvent mixtures.

1.4.2. LSD in illicit preparations

In the illicit market LSD is sold in a variety of forms such as ''microdot'', tablets, gelatine squares or most commonly on impregnated paper sheets. A systematic study of the parameters affecting the LSD extraction efficiency from these sheets has been presented by Veress [29]. Ultrasonic extraction in methanol–water (1:1) of the blotters for 20 min at 20°C and subsequent RP-HPLC separation gave highly reproducible results. HPLC separation was carried out in acetonitrile–phosphate buffer at pH 3.5, UV absorbance detection was at 220 nm.

Kilmer describes extraction of LSD from sugar cubes and from a liquid using ammonium hydroxide and dichloromethane [30]. The dichloromethane was evaporated and methanol was added to the extract. GC–MS analysis then confirmed the presence of LSD.

Ripani et al. describe a method for quantitative capillary GC determination of LSD in street samples [31]. GC with silica Kieselgel was used in the split mode with a 20:1 ratio. After extraction of the LSD with methyl-*tert.*-butyl ether from ''blotters'', the extracts were injected into the capillary GC using triacontane as internal standard. LSD quantitation was possible from 0.03–2 mg/ml when injecting 1 μl of the extract.

2. Phencyclidine (PCP)

2.1. Introduction

Phencyclidine (PCP) is a synthetic drug developed in the 1950s by Parke–Davis and tested as anaesthetic and analgesic. Because of very serious side effects it was removed from the pharmaceutical market and used in veterinary surgery until 1978. In the 1960s PCP became popular as a recreational drug, synthesized in clandestine laboratories. Today, PCP remains a serious problem for a small number of users in some areas of the US (Southern California and Washington DC). It is often smoked with marijuana, either intentionally or unintentionally. Outside the US phencyclidine is a very rare drug.

2.2. Biological effects and pharmacokinetics

The effects of PCP may vary with the dose, the user's settings and previous experiences. Violence and agitation occur in about one third of PCP users [32]. PCP has been reported to have effects on the respiration, the cardiovascular system, the liver and the central nervous system [33,34]. Finally, there is evidence that both physiological and psychological dependence develops [35,36].

It has been suggested that as little as 0.34 ng/ml of PCP in serum may cause psychotic effects. Blood concentrations generally range from 4–100 ng/ml. PCP tends to accumulate in the liver, lungs, brain and the fat tissue of the user [34]. It is then slowly released from fat tissues into the blood stream. Detection in urine is possible up to 7 days after a single dose and as long as 21 days after chronic use.

PCP has been shown to be extensively metabolized by hydroxylation to (4-phenyl-4-piperidino-cyclohexanol, (PPC), 1-(1-phenylcyclohexyl)-4-hydroxypiperidine, (PCHP) [37] or through formation of 5-(N-(1′-phenylcyclohexyl)-amino)pentanoic acid (PCA) [38,39] (Fig. 4). The later is supposed to be the major urinary excretion product.

2.3. Stability

It has been found that PCP concentration in spiked urine samples stored at room temperature may decrease at variable rates [40]. Five out of 15 samples had a >40% decrease in PCP concentration. PCP stability in blood stored at room temperature over a 5 year period also showed a significant decrease in concentration each year. The decrease ranged from 17.6% after 6 months to 69.4% after 5 years [41]. When stored at −16°C to −20°C, no significant loss of PCP in urine was observed [12,42].

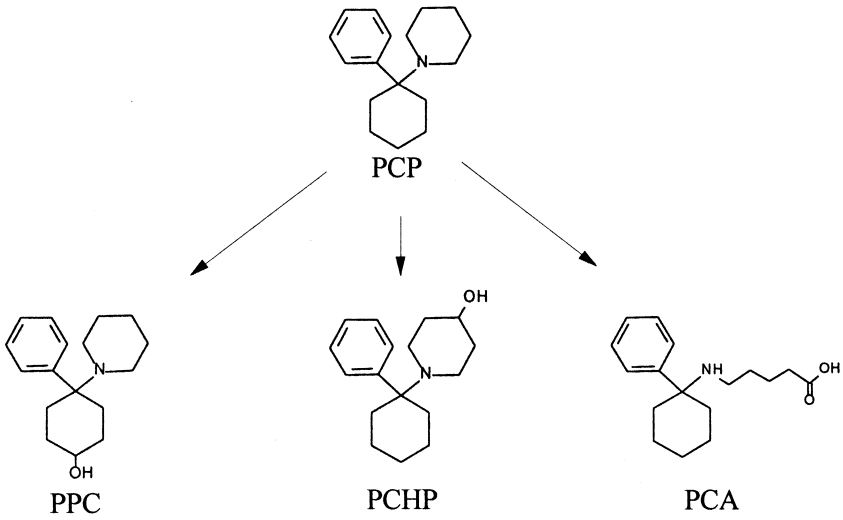

Fig. 4. Structures of phencyclidine (PCP) and its major metabolites.

2.4. PCP analysis in biosamples

PCP in urine or serum specimens is mainly analysed by immunoassay and by GC–MS. Other techniques such as CE and GC with nitrogen–phosphorus detection (GC-NPD) have also been used. Because of interference with other molecules, immunoassays provide qualitative or semiquantitative results only. Quantitation is generally performed using GC–MS in the SIM mode and using an internal standard. The present review describes the detection and quantitation of PCP in biological specimens from 1985 to 1996.

2.4.1. Immunoassays

Because of its speed and simplicity, initial testing of specimens is generally performed by immunoassay. Most authors propose method adaptations or improvements of the commercially available tests. Their aim generally is to lower the cut-off values and/or to obtain faster measurement times [43–54]. Roche [55] and Biosite Diagnostics [56] developed immunoassays based on visual detection of the results. Several authors [57–62] studied the impact of adulterating agents on PCP immunoassays.

An alternative to urine or blood analysis is saliva analysis by immunoassay [63]. It has been found that the concentration of PCP is higher in saliva than in plasma and that PCP analysis in saliva gave more reliable results than PCP analysis in urine because of the stability of the pH in the saliva when compared to urine.

2.4.2. GC–MS

Today GC–MS is the method of choice for screening and confirmation of many toxicants volatile in GC [64]. A typical GC–MS analysis of PCP in urine includes a SPE at pH 6–8; PCP is eluted from the columns with basic ethyl acetate, the extract is evaporated to dryness and the residue is reconstituted in ethyl acetate. Quantitation is generally done by adding an internal standard (pentadeuterated PCP, difluoro-PCP) and working in the SIM mode. The EI–MS spectrum of PCP is shown in Fig. 5. Cleavage of the cyclohexyl ring gives the base peak at m/z 200 [65].

Ishii et al. detected and quantified PCP in whole blood and in urine specimens using GC separation and surface ionisation detection (SID) [66]. Pethidine was used as internal standard. The LOD was 0.75 ng/ml, the quantitation range of spiked samples was 1.25–20 ng/ml

Because of extensive metabolism of PCP, ElSohly et al. [67] developed a method for detection of the PCA metabolite in human urine. The SPE of PCA from urine specimens (5 ml probes) was done using Prep-Sep C_{18} cartridges. Pentadeuterated PCA was used as internal standard. A linear relationship

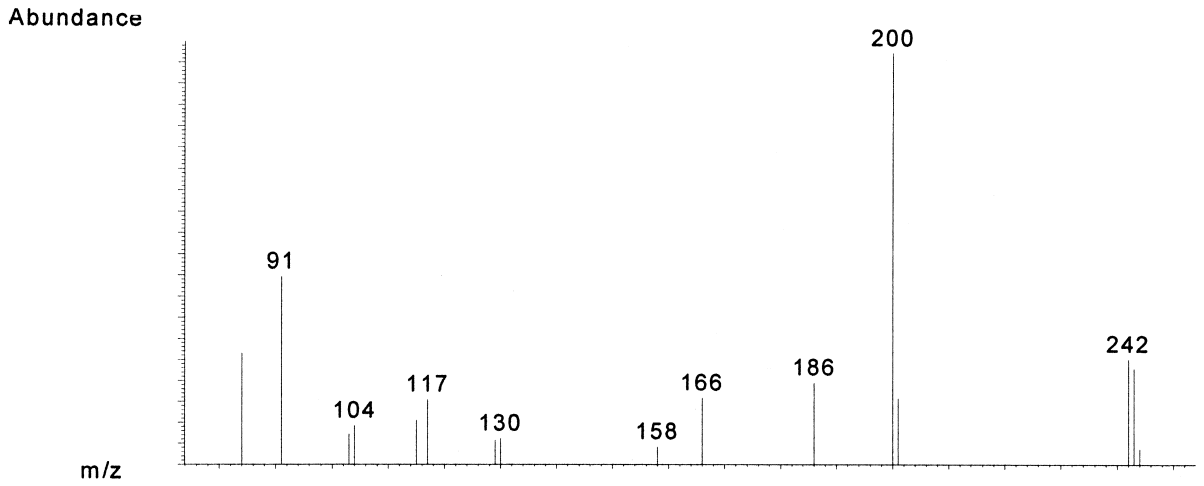

Fig. 5. EI mass spectrum of PCP.

between peak height ratio and PCA concentration was observed over a concentration range from 10–150 ng/ml.

A qualitative and quantitative assay for PCP and other drugs of abuse using SIM was developed by Mulé and Casella [68]. Urine (0.2 ml) spiked with 50 ng of ketamine as internal standard was extracted with chloroform–isopropanol and analysed in the SIM mode after evaporation. A linear relationship between peak height and concentration was observed from 10–100 ng/ml PCP in urine.

Stevenson et al. [69] described a SPE method for PCP in urine. To 5 ml of urine was added d_5-PCP as internal standard and the pH was adjusted to 4.8–5.5. The mixture was then extracted with ammonium hydroxide using Clean Screen™ DAU columns. The extracts were reconstituted with 100 μl of ethyl acetate and GC–MS analysed. The LOD and LOQ were 0.47 and 1.38 ng/ml respectively. Extraction efficiency was found to be 100.8±6.5% and between run precision was 3.5% at 25 ng/ml. According to the authors this method consistently produces cleaner chromatograms than liquid–liquid extraction.

PCP (and other drugs of abuse) where separated and quantified in urine using SPE and ion trap MS following EI ionisation [70]. Difluorophencyclidine was used as internal standard. Recoveries of PCP fluctuated between 90% and 112%. The LOD and LOQ were 0.25 ng/ml and 0.5 ng/ml in the screen mode and 0.25 and 0.32 ng/ml in the SIM mode.

Slawson et al. developed a method for quantitation of PCP in hair using GC–ion trap MS [71]. d_5-PCP was used as internal standard, extraction was carried out with Bond Elut Certify columns. The LOD was 25 pg of PCP, the quantitation range covered 0.1–50 ng/mg of PCP in hair. When comparing the incorporation of PCP into pigmented and nonpigmented hair of rats, it has been found that PCP is incorporated preferentially into the pigmented hair.

A method for the detection of PCP, PCHP and PPC in pigmented rat hair was developed by Sakamoto et al. [65]. The extraction was performed with methanol–5 *M* HCl (20:1). The extract was purified by Bond Elut Certify columns and derivatized with N,O-bis(trimethylsilyl)acetamide. PCP, PCHP-TMS and *trans*-PPC-TMS as well as their deuterium labelled isomers were GC separated; the LODs were determined to be 0.2 ng/mg hair for PCP and 0.04 ng/mg hair for PCHP and *trans*-PPC. It was observed that PCP was incorporated into hair from blood at relatively high rates.

Only PCP but no metabolites was detected in human hair using tandem mass spectrometry [72]. According to the authors, external contamination of hair by PCP appears to be a significant problem and potential source of false positive results.

Two papers describe the detection and quantitation of PCP in meconium. After preliminary immunoassay testing, Moriya et al. [73] extracted the meconium at pH 6 with a Bond Elut Certify column.

The GC–MS was used in the SIM mode and quantitation was achieved using d_5-PCP as internal standard. The LOD was determined to be 20 ng/g. Moore et al. [74] quantified PCP using selected ion storage (SIS) of an ion trap mass spectrometer. SPE was done at pH 3 and deuterated PCP used as internal standard. The LOQ was found to be 5 ng/g. The essential information on GC–MS methods for PCP in biological samples is summarised in Table 1.

2.4.3. Other analytical techniques

NPD has been used for the detection of the nitrogen containing PCP in serum. Detection thresholds ranging 5–150 ng/ml have been described by several authors at the beginning of the 1980s [75–77]. A two-step assay of PCP in serum has been developed by Werner et al. using SPE (Bond Elut-CN column) and GC–NPD [78]. A 2.5 ml volume of serum was required and the LOD of this technique has been determined to be 0.5 ng/ml. The peaks for PCP and the internal standard (methapyrilene) were well separated and no interference with other common drugs of abuse has been observed.

Holsztynska and Domino describe GC–NPD of PCP and its metabolites in tissues, and body fluids of animals and in human urine [79]. After liquid–liquid extraction the extracts were derivatized with heptafluorobutyric acid and injected into the GC–NPD system. The LOD was about 1.2 ng per injection with a linear standard curve to 3.9 ng. The recovery rates of PCP and the metabolites was 80–98%.

Another PCP detection technique using conventional GC–NPD with acetylated column packing material was proposed by Kandiko et al. [80]. Acetylation of Chromosorb W AW-DMCS columns reduces their adsorptive properties towards alkaloids, thus increasing the GC sensitivity. Liquid–liquid extraction of 10 ml urine at pH 6.5–7 was performed with diethyl ether. The LOD of PCP was found to be 15 ng/ml in spiked urine samples. The internal standard used was ketamine. Because of the lack of specificity however all identification of PCP using the NPD technique needs confirmation by another method, for example GC–MS.

CE has been defined as the differential migration of charged species in an electric field. High voltages are used to generate electroosmotic flow of buffer solutions and charged molecules in a column [81]. The molecules are separated as a function of their charge and their size. A detector (UV, MS,...) at the end of the column allows detection and quantitation. CE is a very fast analytical method requiring minute amounts of sample and reagents and is applicable to a wide selection of analytes including stereoisomers.

Chen and Evangelista [82] developed a method allowing simultaneous qualitative and quantitative analysis of multiple drug analytes in urine including PCP. The drug was immunolabeled and then sepa-

Table 1
GC–MS analysis of phencyclidine and metabolites in different biosamples

Analyte	Specimen	Extraction	Derivatization	Internal standard	Detection mode	LOD	ROQ or LOQ	Ref.
PCP	Blood	SPE	–	Pethidine	SID	0.75 ng/ml	1.25–20 ng/ml	[66]
PCP	Urine	SPE	Methylation	d_5-PCA	SIM-EI	nr	10–150 ng/ml	[67]
PCA	Urine	SPE	Methylation	d_5-PCA	SIM-EI	nr	10–150 ng/ml	[67]
PCA	Urine	LLE	–	Ketamine	SIM-EI	nr	10 ng/ml	[68]
PCP	Urine	SPE	–	d_5-PCP	SIM-EI	0.47 ng/ml	1.38–1000 ng/ml	[69]
PCP	Urine	SPE	–	Difluoro-PCP	Full scan	0.25 ng/ml	0.50–500 ng/ml	[70]
PCP	Urine	SPE	–	Difluoro-PCP	SIM-EI	0.25 ng/ml	0.32–500 ng/ml	[70]
PCP	Hair	SPE	–	d_5-PCP	Full scan	nr	0.10–50 ng/ml	[71]
PCP	Hair	SPE	TMS	d_5-PCP	SIM-EI	0.05 ng/mg	0.20–4 ng/mg	[65]
PCHP	Hair	SPE	TMS	d_5-PCHP	SIM-EI	nr	0.04–1 ng/mg	[65]
PCP	Hair	SPE	TMS	d_5-PPC	SIM-EI	nr	0.04–1 ng/mg	[65]
PCP	Hair	–	–	Me-PCP	MS–MS	nr	nr	[72]
PCP	Meconium	SPE	–	d_5-PCP	SIM-EI	20 ng/g	nr	[73]
PCP	Meconium	SPE	–	d_5-PCP	SIS-EI	5 ng/g	5–250 ng/g	[74]

nr: not reported.

rated by CE and detected by laser induced fluorescence. Separation of the analytes takes less than 5 min, the LOD of PCP was found to be 1 ng/ml.

3. Conclusions

PCP as well as LSD remain a significant health problem for a small group of users. Screening of biological specimens for these drugs of abuse is well established for PCP and its major metabolites but remains difficult for LSD.

Immunoassays are prone to adulteration of the specimens or may give false positive results because of interference with other drugs. For LSD, only nonforensic samples may be analysed by immunoassay, HPLC or CE using fluorescence detection. Unambiguous identification can only be provided by chromatographic separation coupled to MS detection. Conventional GC–MS screening however is not sufficient for determination of the very low LSD concentrations in biological specimens. ACU purification coupled to LC–MS working in the SIM mode is the most promising technique. Implantation of an LC–MS system however is costly and needs highly trained manpower. Conducting these assays on a routine daily basis remains a difficult task. Development of this technique may allow us in the future to gain a new insight into LSD metabolism, thus confirming the postulated presence of several human metabolites.

GC–MS is the method of choice for PCP detection in urine and quantitation in blood. Using an internal standard (d_5-PCP, difluoro-PCP) and operating the GC–MS in the SIM mode, allows LOD and LOQ in the ng/ml range. Alternatively, other techniques such as tandem MS, NPD and CE have been used. Each of these methods has its advantages and disadvantages.

4. Abbreviations

ACU	affinity clean-up
CE	capillary electrophoresis
CE–MS	capillary electrophoresis–mass spectrometry
CI	chemical ionisation
EI	electron impact
ESI	electrospray ionisation interface
ESI–MS	electrospray ionisation–mass spectrometry
GC–MS	gas chromatography–mass spectrometry
GC–NPD	gas chromatography–nitrogen–phosphorus detection
HPLC	high-performance liquid chromatography
LAE	lysergic acid ethylamide
LAMPA	lysergic acid methylpropylamide
LC–MS	liquid chromatography–mass spectrometry
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LSD	lysergic acid diethylamide (from the German: Lysergsäure-diethylamid)
PCA	5-(N-(1′-phenylcyclohexyl)-amino)-pentanoic acid
PCHP	1-(1-phenylcyclohexyl)-4-hydroxypiperidine
PCP	phencyclidine, 1-(1-phenylcyclohexyl)-piperidine
PPC	4-phenyl-4-piperidinocyclohexanol
RIA	radioimmunoassay
RP-HPLC	reverse phase high-performance chromatography
SID	surface ionisation detection
SIM	selected ion monitoring
SIS	selected ion storage
SPE	solid-phase extraction
TFA	trifluoroacetyl
TMS	trimethylsilyl

References

[1] A. Hofmann, Die Mutterkornalkaloide [The Ergot Alkaloids], F. Enke Verlag, Stuttgart, 1964.
[2] A. Stoll, A. Hofmann, Z. Physiol. Chem. 251 (1938) 155.
[3] A. Hofmann, LSD: Mein Sorgenkind [LSD: My Problem Child], Klett-Cotta/DTV, 1993.
[4] NIDA, Annual National High School Senior Survey, Rockville, MD, April 13, 1993.
[5] F. Musshoff, T. Daldrup, Forensic Sci. Int. 88 (1997) 133.
[6] B. Jacobs, M. Trulson, J. Heym, Brain Res. 215 (1981) 275.
[7] R. Glennon, Neuropsychopharmacology 3 (1990) 509.
[8] H.K. Lim, D. Andrenyak, P. Francom, R.T. Jones, R.L. Foltz, Anal. Chem. 60 (1988) 1420.
[9] C.C. Nelson, R.L. Foltz, J. Chromatogr. 580 (1992) 97.

[10] J. Cai, J. Henion, J. Anal. Toxicol. 20 (1996) 27.
[11] Z.H. Ziddik, R.D. Barnes, L.G. Dring, R.L. Smith, R.T. Williams, Biochem. Pharmacol. 28 (1979) 3081.
[12] B.D. Paul, R.M. McKinley, J.K. Walsh Jr., T.S. Jamir, M.R. Past, J. Anal. Toxicol. 17 (1993) 378.
[13] B.D. Paul, J.M. Mitchell, R. Burbage, M. Moy, R. Sroka, J. Chromatogr. 529 (1990) 103.
[14] K.S. Webb, P.B. Baker, N.P. Cassells, J.M. Francis, D.E. Johnston, S.L. Lancaster, P.S. Minty, G.D. Reed, S.A. White, J. Forensic Sci. 41 (1996) 938.
[15] A.J. McNally, Z. Li, I. Pilcher, S.J. Salamone, Abstracts of XXXV Annual TIAFT Meeting, Padova, August 1997, Padova, 1997, p 17.
[16] R. Gill, J.A. Key, J. Chromatogr. 346 (1985) 423.
[17] M. Japp, R. Gill, M.O. Osselton, J. Forensic Sci. 32 (1987) 933.
[18] A.J. McNally, K. Goc-Szkutnicka, Z. Li, I. Pilchner, S. Polakowski, S.J. Salamone, J. Anal. Toxicol. 20 (1996) 404.
[19] A.H. Wu, Y.-J. Feng, A. Pajor, T.G. Gornet, S.S. Wong, E. Fort, J. Brown, J. Anal. Toxicol. 21 (1997) 181.
[20] A. Verstraete, T. Troch, in: TIAFT Proceedings of the 34th Triennial Meeting, Interlaken, August 11–15, 1996, in press.
[21] N.P. Cassells, D.H. Craston, C.W. Hand, D. Baldwin, J. Anal. Toxicol. 20 (1996) 409.
[22] J.M. Francis, D.H. Craston, Analyst 121 (1996) 177.
[23] S.A. White, T. Catterick, M.E. Harrison, D.E. Johnston, G.D. Reed, K.S. Webb, J. Chromatgr. B. 689 (1997) 335.
[24] Y. Nakahara, R. Kikura, K. Takahashi, R.L. Foltz, T. Mieczkowski, J. Anal. Toxicol. 20 (1996) 323.
[25] C.C. Nelson, R.L. Foltz, Anal. Chem. 64 (1992) 1578.
[26] G.S. Rule, J.D. Henion, J. Chromatogr. 582 (1992) 103.
[27] G. Hopfgartner, T. Wachs, K. Bean, J. Henion, Anal. Chem. 65 (1993) 439.
[28] H. Hoja, P. Marquet, B. Verneuil, H. Lofti, J.L. Dupuy, G. Lachâtre, J. Chromatogr., in press.
[29] T. Veress, J. Forensic Sci. 38 (1993) 1105.
[30] S.D. Kilmer, J. Forensic Sci. 39 (1994) 860.
[31] L. Ripani, S. Schiavone, L. Garofano, J. Forensic Sci. 39 (1994) 512.
[32] M.M. McCarron, NIDA Res. Monogr. Ser. 64 (1986) 209.
[33] F.N. Pitts, R.E. Allen, O. Aniline, L.S. Yago, Clin. Toxicol. 18 (1981) 964.
[34] M. Johnstone, V. Evans, Br. J. Anaesth. 31 (1959) 433.
[35] K.B. Yago, F.N. Pitts Jr., R.W. Burgoyne, O. Aniline, L.S. Yago, A.F. Pitts, J. Clin. Psychiatry 42 (1981) 193.
[36] R.L. Balster, NIDA Res. Monogr. Ser. 64 (1986) 148.
[37] L.K. Wong, K. Biemann, Clin. Toxicol. 9 (1976) 586.
[38] L.S. Cohen, L. Gosenfeld, J. Wilkins Jr., New Engl. J. Med. 306 (1982) 1427.
[39] C.D. Syracuse, B.R. Kuhnert, N.L. Golden, B.S. Bagby, Biomed. Environ. Mass Spectrom. 13 (1986) 113.
[40] R. Hughes, A. Hughes, B. Levine, M.L. Smith, Clin. Chem. 37 (1991) 2141.
[41] S.N. Giorgi, J.E. Meeker, J. Anal. Toxicol. 19 (1995) 392.
[42] S. Dugan, S. Bogema, R.W. Schwartz, N.T. Lappas, J. Anal. Toxicol. 18 (1994) 391.
[43] V.R. Spiehler, P. Sedgwick, J. Anal. Toxicol. 9 (1985) 63.
[44] T.A. Appel, N.A. Wade, J. Anal. Toxicol. 13 (1989) 274.
[45] Y.H. Caplan, B. Levine, J. Anal. Toxicol. 13 (1989) 289.
[46] P.L. Cary, C.A. Johnson, T.M. Folsom, W.R. Bales, J. Anal. Toxicol. 16 (1992) 48.
[47] M.A. ElSohly, D.F. Stanford, J. Anal. Toxicol. 14 (1990) 192.
[48] R.A. Klinger, L.M. Blum, F. Rieders, J. Anal. Toxicol. 14 (1990) 288.
[49] T.C. Sneath, N.C. Jain, J. Anal. Toxicol. 16 (1992) 107.
[50] W.M. Asselin, J.M. Leslie, J. Anal. Toxicol. 16 (1992) 381.
[51] D.T. Diosi, D.C. Harvey, J. Anal. Toxicol. 17 (1993) 133–137.
[52] R.C. Sreenivasam, T.C. Sneath, N.C. Jain, J. Anal. Toxicol. 17 (1993) 370.
[53] M.J. Fyfe, P. Chand, C. McCutchen, J.S. Long, A.S. Walia, J. Anal. Toxicol. 17 (1993) 188.
[54] R.G. Parsons, R. Kowal, D. LeBlond, V.T. Yue, L. Neargarder, L. Bond, D. Garcia, D. Slater, P. Rogers, Clin. Chem. 39 (1993) 1899.
[55] D.A. Armbruster, J.M. Krolak, J. Anal. Toxicol. 16 (1992) 172.
[56] A.H. Wu, S.S. Wong, K.G. Johnson, J. Callies, D.X. Shu, W.E. Dunn, S.H.Y. Wong, J. Anal. Toxicol. 17 (1993) 241.
[57] S.D. Pearson, K.O. Ash, F.M. Urry, Clin. Chem. 35 (1989) 636.
[58] J.T. Cody, R.H. Schwarzhoff, J. Anal. Toxicol. 13 (1989) 277.
[59] W. Bronner, P. Nyman, D. von Minden, J. Anal. Toxicol. 14 (1990) 368.
[60] R. Schwarzhoff, J.T. Cody, J. Anal. Toxicol. 17 (1993) 14.
[61] A. Warner, Clin. Chem. 35 (1989) 648.
[62] B.S. Levine, M.L. Smith, Clin. Chem. 36 (1990) 1258.
[63] W. Schramm, R.H. Smith, P.A. Craig, D.A. Kidwell, J. Anal. Toxicol. 16 (1992) 1.
[64] H.H. Maurer, J. Chromatogr. Biomed. Appl. 580 (1992) 3.
[65] T. Sakamoto, A. Tanaka, Y. Nakahara, J. Anal. Toxicol. 20 (1996) 124.
[66] A. Ishii, H. Seno, T. Kumazawa, M. Nishikawa, K. Wanatabe, O. Suzuki, Int. J. Legal Med. 108 (1996) 244.
[67] M.A. ElSohly, T.L. Little Jr., J.M. Mitchell, B.D. Paul, L.D. Mell Jr., J. Irving, J. Anal. Toxicol. 12 (1988) 180.
[68] S.J. Mulé, G.A. Casella, J. Anal. Toxicol. 12 (1988) 102.
[69] C.C. Stevenson, D.L. Cibull, G.E. Platoff Jr., D.M. Bush, J.A. Gere, J. Anal. Toxicol. 16 (1992) 337.
[70] A.H. Wu, T.A. Onigbinde, S.S. Wong, J. Anal. Toxicol. 16 (1992) 202.
[71] M.H. Slawson, D.G. Wikins, R.L. Foltz, D.E. Rollins, J. Anal. Toxicol. 20 (1996) 350.
[72] D.A. Kidwell, J. Forensic Toxicol. 38 (1993) 272.
[73] F. Moriya, K.-M. Chan, T.T. Noguchi, P.Y.K. Wu, J. Anal. Toxicol. 18 (1994) 41.
[74] C.M. Moore, D.E. Lewis, J.B. Leikin, J. Forensic Sci. 41 (1996) 1057.
[75] S. Stavchansky, A. Loper, J. Pharm. Sci. 71 (1982) 194–196.
[76] D.N. Bailey, J.J. Guba, Clin. Chem. 26 (1980) 434.
[77] F.N. Pitts Jr., L.S. Yago, O. Aniline, A.F. Yago, J. Chromatogr. 193 (1980) 157.

[78] M. Werner, M. Herzman, C.J. Pauley, Clin. Chem. 32 (1986) 1921.
[79] E.J. Holsztynska, E.F. Domino, J. Anal. Toxicol. 10 (1986) 107.
[80] C.T. Kandiko, S. Browning, T. Cooper, W.A. Cox, J. Chromatogr. 528 (1990) 208.
[81] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995.
[82] F.-T.A. Chen, R.A. Evangelista, Clin. Chem. 32 (1994) 1819.