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Study of the Extraction of LSD from Illicit Blotters for HPLC Determination

REFERENCE: Veress, T., **"Study of the Extraction of LSD from Illicit Blotters for HPLC Determination,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 5, Sept. 1993, p. 1105–1110.

ABSTRACT: The effect of different parameters (extraction method, temperature, time, solvent) on the extraction efficiency of LSD from impregnated papers has been investigated. Designed experiments have been applied according to the Plackett-Burman method. For the quantitative determination of LSD reversed phase ion-pair chromatography has been used with UV detection. The experimental conditions assuring maximal LSD recovery have been determined, as well as the constant and proportional bias of the extraction procedure elaborated.

KEYWORDS: forensic science, lysergic acid diethylamide (LSD), experimental design, chromatographic determination, extraction

(+)-lysergic acid diethylamide (LSD) is one of the most potent hallucinogenic substances. At the present time, the great majority of the types of LSD dosage forms found in the illicit market are the impregnated paper sheets. The content of these forms is generally between 30 μg and 500 μg of LSD. Forensic-science laboratories require fast, accurate and reliable analytical procedures to identify and quantify the LSD in exhibits mentioned. For the positive identification of LSD the application of mass spectrometry [*1*] or infrared spectroscopy [*2*] are preferred. Bowen and co-workers [*3*] used circular dichroism spectropolarimetry for the quantitative determination of LSD in confiscated materials without preliminary separation. Other authors prefer chromatographic methods [*4–7*] for the quantitative determination of LSD, especially the application of high performance liquid chromatography (HPLC) because of the low volatility of the compound.

The reliability of the determination of LSD is highly affected by the sample preparation, especially, by the extraction, applied prior to the chromatographic separation. As far as the extraction solvent and extraction method are concerned it is not clear which is the best solvent and extraction method. Methanol [*5,6,8,9*], water [*9*], aqueous solutions of tartaric acid [*8,10*], hydrochloric acid [*3,11*], sulphuric acid [*12*] and mixtures of methanol-water (1:1) solvent mixture [*4,13*] were also used for the extraction of LSD from illicit preparations. Both ultrasonic vibration [*13*] and soaking [*11*] were applied for the extraction.

We have studied the extraction of LSD from blotters investigating the effect of different variables (extraction method, temperature, time, solvent) on the extraction efficiency. Statistically designed experiments were applied according to the Plackett-Burman [*14*] method in order to select the experimental conditions assuring maximal LSD re-

Received for publication 5 Nov. 1992; revised manuscript received 22 Feb. 1993; accepted for publication 24 Feb. 1993.

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covery. The constant and proportional errors of the procedure have also been determined. For the quantitative tracing of LSD reversed phase ion-pair chromatography was applied.

Experimental

Materials, Equipments

The methanol and acetonitrile used for the extraction and HPLC separation, respectively were Lichrosolv-grade (Merck). The water was purified according to Gurkin's method [*15*] on a reversed phase preparative column type of Hybar (Merck).

The LSD standard was received from the UN Narcotic Laboratory Section, Vienna, as 0.1 mg/mL LSD tartrate in methanolic solution. The LSD blotters used for the experiments were seized by the Hungarian drug enforcement agencies. The LSD-free, blank blotters were prepared by removing the LSD from blotters by multiple extraction.

The extraction experiments were performed by using a Reacti-Therm heating module (Pierce) or a KLN G40/41 (KLN Ultraschall GmbH) ultrasonic bath, respectively.

The HPLC separation was carried out on a Hewlett Packard 1084B liquid chromatograph equipped with a HP 79875A variable UV detector and a HP 79850B computing integrator.

The mass spectrometric identification was carried out on a Hewlett Packard 5985 GC/MS system.

HPLC Conditions

Mobile phase: acetonitrile-phosphate buffer (45:55 v/v).
Phosphate buffer: 0.05M KH_2PO_4 and 0.005M $C_8H_{17}SO_3Na$ containing aqueous solution, pH 3.5.
Column: Zorbax ODS, 25 cm × 4.6 mm I.D .8 μm particle size.
Flow: 0.9 mL/min.
Temperature 60°C.
Detection: 220 nm.
Injection: 20 μL.

The LSD peak was identified by comparison of its retention time with that of standard. A typical chromatogram of an LSD blotter extract prepared with methanol is shown in Fig. 1. A good separation could be achieved between the extract components within 8 minutes. The identification of the LSD peak was confirmed by mass spectrometric analysis of the appropriate collected effluent fraction.

For the quantitative determination external standard method was applied by evaluation of the HPLC peak areas.

Experimental Design

Effects of the extraction method, temperature, time and solvent type on the extraction of LSD from blotters were studied by experimental design methods using two-level eight experiment designs.

The evaluation was performed according to calculation of the effect (E) of variables. The effect for a given variable can be calculated by the following expression.

$$E = \left(\sum_{i=1}^{k} R_i^{+} + - \sum_{i=1}^{k} R_i^{-} \right) / (k/2),$$

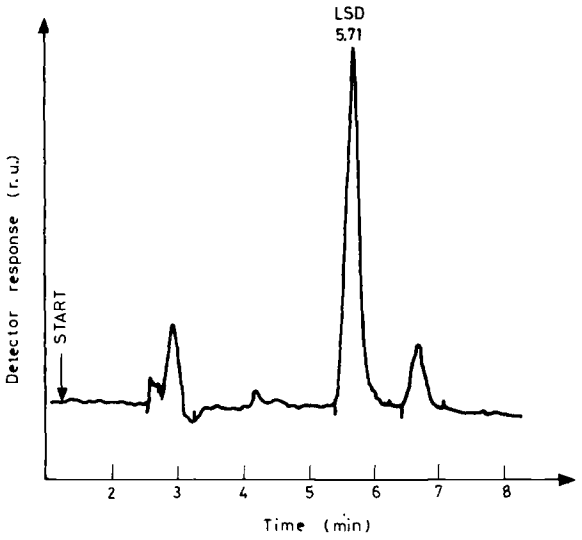

FIG. 1—*A typical HPLC chromatogram of an LSD blotter extract. (For chromatographic conditions see text.)*

where R^+ and R^- are the experimental results obtained from experiments, where the variable was set at high and low level, respectively and k is the number of experiments. The results were evaluated by the so-called half-normal plotting of the effects calculated, according to Daniel's method [*16*]. The method is based on that assumption that the effect of those variables,which do not affect significantly the investigated process, has a normal distribution. The distribution of effects, which are actually important will deviate from the normal distribution.

Extraction Experiments

For the study of the extraction efficiency of LSD from blotters, 113±3 μg LSD containing blotters were used as test material. The homogeneity and the identical composition of the blotters with each other were previously confirmed by eleven parallel HPLC measurements of the blotters from the batch, applying multiple extraction.

The ultrasonic extraction was performed in glass test tubes. For the quasi countercurrent extraction the blotters were inserted into a glass extraction column (5 cm × 8 mm I.D.) between two glass fiber beds and solvent was delivered through with a peristaltic pump. The extraction column was thermostated to a heating module and the extraction solvent was warmed up to the set temperature prior to the run.

The extractions were done with 5 mL of solvent and the resulted solutions were diluted to 10 mL with the same solvent as used for the extraction. The other conditions for the designed experiments are listed in Table 1.

Recovery Experiments

In order to determine the corrigible systematic errors of the extraction procedure LSD-free blotters were spiked with known amounts of LSD in the range of 40 to 300 μg and the reextracted amounts of LSD were determined by HPLC. The conditions of the extraction are given later as conditions suggested for routine analysis.

TABLE 1—*Values of variables set for the study of extraction of LSD.*

Code	Variable	Low level (−)	High level (+)
A.	Extraction method	ultrasonic	quasi counter-current
B.	Temperature	20°C	60°C
C.	Extraction time	20 min	30 min
D.	Solvent	methanol-water 1:1	methanol
E.	Dummy[a]	no change	no change
F.	Dummy[a]	no change	no change
G.	Dummy[a]	no change	no change

[a]The effects of the dummy variables can be used for the calculation of random error.

Results and Discussion

The plan of the Plackett-Burman design applied for the study of extraction of LSD and typical results of the experiments are shown in Table 2. The effects calculated from the experimental results are listed in Table 3. The largest effect was caused by changing the solvent type, which was followed by the extraction time and method. The alteration of the extraction temperature had the least effect. The positive sign of the effects indicates that the extraction at the high level of the variable resulted in a greater amount of extracted LSD. The results were graphically evaluated by half-normal plotting of the rank probabilities against the variable effects, as described Daniel [*16*]. A typical plot is shown in Fig. 2. In the figure there is only one point, which significantly deviates from the straight

TABLE 2—*Plackett-Burman design for screening seven variables in eight experiments, with the experimental results.*

	Variables[a]							Experimental results
No.	A	B	C	D	E	F	G	LSD (μg)
1	+	+	+	−	+	−	−	115.5
2	−	+	+	+	−	+	−	95.6
3	−	−	+	+	+	−	+	96.6
4	+	−	−	+	+	+	−	78.5
5	−	+	−	−	+	+	+	111.6
6	+	−	+	−	−	+	+	110.3
7	+	+	−	+	−	−	+	86.5
8	−	−	−	−	−	−	−	113.7

[a]The variables listed in Table 1.

TABLE 3—*Effects of variables on the extraction efficiency of LSD. (Calculated from results given in Table 2.)*

Code	Variable	Effect (μg)
A.	Extraction method	−6.7
B.	Temperature	2.5
C.	Extraction time	6.9
D.	Solvent	−23.5
E.	Dummy[a]	−1.0
F.	Dummy[a]	−4.1
G.	Dummy[a]	0.4

[a]The effects of the dummy variables can be used for the calculation of random error.

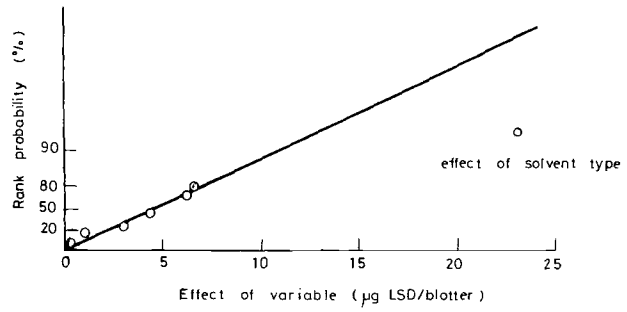

FIG. 2—*Half-normal plot for the evaluation of effects of variables affecting the extraction of LSD.*

line representing the normal distribution standard error. This point represents the effect of solvent type, which significantly affects the extraction of LSD. As the sign of the effect of solvent type is negative, the extraction efficiency is better at the low level of this variable, that is, methanol-water (1:1) mixture is more effective for the extraction of LSD than the pure methanol is. This finding can easily be accepted by considering that the blotters are generally impregnated by water-soluble salts of LSD, having less solubility in pure alcohols than in aqueous solutions. As Fig. 2 shows, the other variables do not affect significantly the efficiency of the extraction of LSD. Their effects are in the range of experimental error. In this manner for routine analysis the ultrasonic extraction of the blotters with 5 mL methanol-water (1:1) solvent mixture for 20 min at ambient temperature can be suggested.

By setting the extraction conditions as mentioned above the relative standard deviation ($n=8$) of the procedure was within 4%.

The results of the recovery experiments are shown in Fig. 3, where the reextracted LSD amounts are plotted against the spiked amounts. A good linearity is established between the recovered and added LSD amounts. For the slope (proportional error) 0.96 and for the intercept (constant error) 0.01 values were calculated, respectively. According to these values the systematic error of the determination caused by the extraction waste can be eliminated.

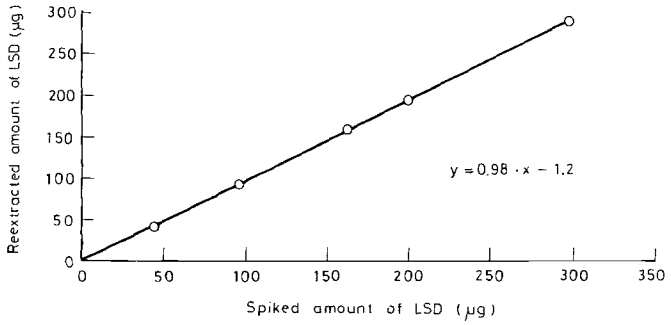

FIG. 3—*Plot of reextracted amounts of LSD against spiked amounts. (The spiked blotters were sonicated with 5 mL MeOH-HOH (1:1) solvent mixture for 30 min at 60°C.*

Conclusion

With methanol-water (1:1) solvent mixture a significantly higher efficiency can be achieved for the extraction of LSD from paper blotters than with pure methanol. Both the one-step ultrasonic extraction and quasi counter-current extraction for 20 to 30 minutes are satisfactory either at 20°C or at 60°C. In routine work, applying ultrasonic extraction of the blotters with 5 mL methanol-water for 20 min at 20°C and a subsequent reversed phase ion-pair HPLC separation, the relative standard deviation of the LSD determination was not greater than 4%. The systematic errors (constant and proportional) of the extraction procedure have also been determined, allowing reliable quantification of the LSD. The proposed procedure is simply and rapidly applicable for the routine determination of LSD in blotters.

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