Analysis of a New Potent Hallucinogen, 25-B-NBOMe, in Blotters by High-Performance Thin-Layer Chromatography

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Key Words

High-performance thin-layer chromatography
25-B-NBOMe
Blotters
Hallucinogens
Validation
Quantitation

1 Introduction

All over the world, hallucinogens have long been abused to experience their effects; in this context, abuse of drugs is a serious public health issue. Because the consumption of blotters impregnated with drug is very frequent, hallucinogens are the most common drugs found in blotters [1]. Since decades, blotters have been generally impregnated with the potent hallucinogen known as lysergic acid diethylamide (LSD). This substance is considered as a low toxic hallucinogen; however, for a couple of years, numerous blotters have been seized in Chile and other countries with *N*-(*2-methoxy)benzyl*-substituted phenylethylamine hallucinogens, designated as NBOMe derivatives [2]. The structural analogues of substituted phenylethylamine are a group of newly synthesized chemical compounds and, according to scientific studies, demonstrate a high affinity for 5HT2A receptor; international reports indicate that it even could be higher than the LSD [3]. These new drugs are represented essentially by three compounds known as 25 I NBOMe, 25 C NBOMe, and 25 B NBOMe if they contain iodine, chlorine, or bromine in their structure (**Figure 1** shows these chemical structures) [4]. Numerous reports of deaths and hospitalizations related to the consumption of these new synthetic compounds impregnated in blotters have occurred in the United States and Europe [5, 6]. In this situation and in order to determine the content of 25-B-NBOMe, we implemented and validated a fast and reliable method for the quantitative analysis of 25-B-NBOMe in seized blotters by high-performance thin-layer chromatography (HPTLC) which offers comparative advantages over other methods previously implemented.

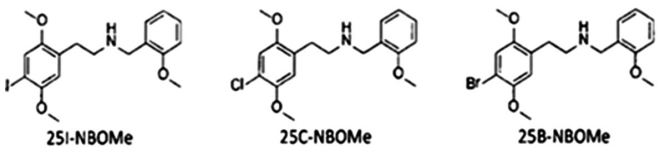

Figure 1

Chemical structures of the most representative N-(2-methoxy)benzyl-substituted phenylethylamine derivatives.

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2 Experimental

2.1 Chemicals and Reagents

Ammonia, methanol, toluene, diethylamine, and cyclohexane were high-performance liquid chromatography (HPLC) grade and were purchased from Merck (Darmstadt, Germany). Reference material of 25-B-NBOMe was kindly provided by the Drug Enforcement Administration DEA (Washington, DC).

2.2 Sample Preparation

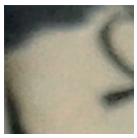
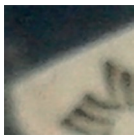
The extraction procedure was carried out by submerging one blotter into 25.0 mL of methanol (HPLC grade), then extracted in ultrasonic bath for 15 min;an amount of 2.0 mL was added to a glass vial for HPTLC analyses. The results and design of each analyzed blotter are shown in **Table 1.**

2.3 Chromatography

HPTLC was performed on 20 × 10 cm precoated silica gel F_{254} plates (Merck, Darmstadt, Germany) previously activated at 80°C for 30 min. Standards (2 µL) and samples (2 µL) were applied in 3 mm bands with an ATS 4 automatic TLC sampler (CAMAG, Muttenz, Switzerland), using a spray band technique. During the first application, the *x* axis was 15 mm and the *y* axis was 8.0 mm, and the distance between tracks was 5.8 mm. The plates were developed in an automatic developing chamber (ADC-2, CAMAG) to a distance of 70 mm with cyclohexane–toluene–diethylamine (75:15:10) as the mobile phase (10 mL, without saturation of the chamber). After a drying time of 5.0 min, the bands were scanned with a CAMAG TLC Scanner 4 densitometer by absorbance at 298 nm **(Figure 2).** The spectrum of each peak was recorded in the 190–400 nm range

Table 1

Results of analysis of five real samples containing 25-B-NBOMe.

Sample name	Amount of 25-B-NBOMe (µg/blotter)	Design
3117-1	1621.5	
3117-2	1621.1	
3247	935.25	
3118-1	1203.75	
3118-2	950.88	

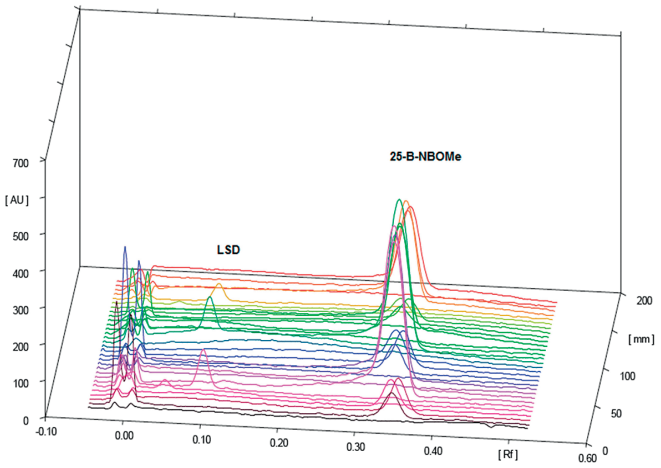


Figure 2

Densitogram of 25-B-NBOMe (R_F 0.34) and LSD (R_F 0.06) at 298 nm.

on all detected peaks mode; slit dimension, 4.00 × 0.30 mm; scanning speed, 20 nm s^{-1}; data resolution, 100 µm $step^{-1}$; and reference spectrum, x = 10.0 mm, y =5.0 mm, and it was controlled with winCATS Planar Chromatography Manager version 1.4.7 software (CAMAG).

3 Results and Discussion

The implemented method was validated according to the recommendations of SWGTOX [7]. In terms of selectivity, we found an acceptable correlation between the UV spectra acquired from the standard and from real samples, and no other peaks were present at the R_F of 25-B-NBOMe. Concerning specificity, TLC plates from another manufacturer (Macherey-Nagel, Düren, Germany) did not alter the separation process. The linearity of the method was evaluated from the calibration plot constructed by analysis of six independent solutions prepared by dissolving appropriate amounts of 25-B-NBOMe to obtain concentrations from 19.18 to 115.00 µg per band; each point was applied in triplicate. Data were fitted by the linear equation $y = 14.24x + 43.19$, and the coefficient of determination (R^2) was 0.9963. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the formulas LOD = 3.3xSa/b and LOQ = 10xSa/b [8], where Sa is the standard deviation of the intercept and b is the slope of the calibration curve [9]; in this case, LOD was 7.12 µg per band and LOQ was 21.56 µg per band. Precision of the method was evaluated in terms of relative standard deviation (RSD) under conditions of repeatability and reproducibility (intermediate precision). RSD of repeatability (intra-day) was obtained by analyzing ten independent replicates of one sample on the same day and by the same analyst; the result of repeatability was 2.77%. The intermediate precision was evaluated by quantifying six independent levels of concentration on three different days by the same analyst, and the result was 6.26%. The method accuracy was 97.57% and was measured by determination of the percentage of 25-B-NBOMe recovered by the assay at three different levels. In order to estimate the relative uncertainty (U%), we used the results from method validation and then U% was calculated with the formula:

$$U\% = \sqrt{CVrepeat^2 + CVrecover^2 + CVreprod^2},$$

where CVrepeat is the relative standard deviation of repeatability, CVrecover is the relative standard deviation of the accuracy assay, and CVreprod is the relative standard deviation from the intermediate precision [10]. The relative expanded uncertainty was 8.41%. In the case of robustness, different HPTLC plates from another manufacturer (Macherey-Nagel) did not modify the separation process. With the validated method, we analyzed five real samples of blotters (size 1.0 cm × 1.0 cm) seized by the Chilean Police in 2014.

4 Conclusion

A new HPTLC method was developed and fully validated for qualitative and quantitative analysis of 25-B-NBOMe in seized blotters. Results of validation parameters were in the accepted range according to SWGTOX recommendations, and thus the proposed method appears to be fast, precise, and reliable for the determination of the content of this compound in seized blotters, with low cost of development and very small sample manipulation. This method can easily be implemented in forensic laboratories. The results obtained are alarming, bearing in mind the high potency of 25-B-NBOMe, particularly when the customer is not aware about the substance impregnated in the blotter, because in general, blotters are usually impregnated with hallucinogens such as lysergic acid diethylamide (LSD); however, at present, other harmful psychoactive substances are detected. Another important issue is the range of concentration of 25-B-NBOMe (935.25 to 1621.5 µg per blotter); this detail is alarming from the point of view

of toxicology and due to the dangerous effects of this substance. To the best of our knowledge, this is the first report on analytical methods for testing 25-B-NBOMe in seized blotters by HPTLC.

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