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Short Communication

Development of a Thin Layer Chromatography Method for the Qualitative Detection of Pregabalin in Human Urine

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ABSTRACT

Background: Pregabalin (PGB) misuse represents a growing public health concern in Algeria, where routine urinary immunoassay screening is limited by elevated cut-off values (≥ 500 µg/L) and cross-reactivity with structurally related compounds, notably gabapentin. This study aimed to develop and validate a simple, low-cost thin-layer chromatography (TLC) method for the qualitative detection of pregabalin in human urine.

Methods: Urine samples were acidified, extracted with ethyl acetate, and spotted onto silica gel plates. Development used a methanol–ammonia mobile phase (98.5:1.5, v/v), and detection was achieved by spraying with ninhydrin reagent followed by heating at 60°C, yielding a characteristic violet spot at Rf = 0.56.

Results: The method demonstrated excellent selectivity (6/6 blank matrices), full specificity against a panel of 30 drugs and substances of abuse, a limit of detection (LOD) of 100 µg/L, and urine stability at −20°C for seven days. Applied to 87 clinical samples, TLC identified 10 false-negative immunoassay results and resolved 7 gabapentin-related false-positive cases, confirming its diagnostic value in resource-limited toxicology settings.

Conclusion: This validated, chloroform-free TLC method provides a cost-effective, selective, and highly sensitive alternative for urinary pregabalin screening, making it an ideal tool for resource-limited toxicology laboratories.

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Introduction

Pregabalin (PGB), chemically designated as (S)-3-(aminomethyl)-5-methylhexanoic acid, is a synthetic analogue of gamma-aminobutyric acid with a molecular formula of C8H17NO2 and a molecular weight of 159.23 g/mol [1]. Following oral ingestion, it is rapidly absorbed, reaching peak plasma concentrations within one hour, and is eliminated almost entirely by the kidneys in its unchanged form, with a plasma half-life of approximately six hours [2, 3]. Pharmacologically classified as an anticonvulsant, PGB is commonly prescribed for partial-onset seizures, neuropathic pain, and generalized anxiety disorder [4]. Beyond its therapeutic applications, it is increasingly misused for the euphoric and dissociative sensations it produces, as well as its substantial capacity to induce tolerance and dependence [5]. In Algeria, its formal reclassification as a controlled psychotropic substance through an interministerial decree published in the Official Journal No. 61 of August 2021 reflected the growing magnitude of this public health threat.

The epidemiological burden of pregabalin misuse in Algeria has escalated sharply over recent years. Surveillance data from the Ouargla region, spanning 2017 to 2023, documented 152 pregabalin-related intoxication cases, representing 10.8% of all recorded intoxications (n = 1,410) and nearly half (44.6%) of those attributed to psychoactive substances [6]. The temporal trend was particularly striking, with the proportion rising from 0.8% in 2017 to 21.5% by 2023. Data from the Toxicology Department of CHU Mohamed Lamine Debaghine, Algiers, further documented a 300% surge in female pregabalin addiction cases between 2021 and 2022, with polysubstance use being the predominant clinical pattern [7]. Fatal outcomes attributable to pregabalin, whether consumed in isolation or combined with other psychoactive agents, have also been increasingly reported in the forensic literature.

Routine urinary screening for PGB relies predominantly on rapid immunoassay strip tests. However, these tests are hampered by inherently elevated detection thresholds, with cut-off values typically ranging from 500 to 1000 µg/L, and by susceptibility to cross-reactivity with structurally related compounds such as gabapentin, generating clinically significant false-positive and false-negative results [8, 9]. Confirmatory hyphenated techniques, notably liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–mass spectrometry (GC-MS), offer substantially superior analytical performance and specificity, but their deployment is restricted by prohibitive equipment and maintenance costs, rendering them inaccessible to most routine toxicology laboratories in resource-limited settings [10]. Thin-layer chromatography (TLC) represents a validated, low-cost, and technically accessible analytical alternative for toxicological screening. However, only one prior study has documented urinary pregabalin detection by TLC [11], and this method presents significant limitations: it relies on chloroform as a mobile phase component, a solvent with well-documented hepato- and nephrotoxic properties [12] and no validation parameters were reported. No validated, chloroform-free TLC method for urinary pregabalin detection has previously been described.

Materials and Methods

Three millilitres of urine were acidified by adding 50 µL of 3N hydrochloric acid, then subjected to liquid–liquid extraction using 8 mL of ethyl acetate. Following vortex agitation and centrifugation at 4500 rpm for five minutes, the organic supernatant was carefully collected and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was reconstituted in 500 µL of methanol, and an aliquot of 50 µL was deposited onto silica gel 60 F_{254} TLC plates (5 × 10 cm, Merck®). Chromatographic development was performed in a glass chamber pre-saturated with the mobile phase, consisting of a methanol–ammonia mixture (98.5:1.5, v/v). After complete development and air-drying, plates were examined under UV light at both 254 nm and 366 nm. Colourimetric detection was then achieved by uniformly spraying the plates with a 1% ninhydrin reagent, followed by brief heating at 60°C. GC-MS analysis, performed on a Shimadzu GC-201 PLUS® coupled to a GC-MS-QP2020 NX® quadrupole mass spectrometer (electron impact, 70 eV), served as the reference confirmatory method throughout the study.

Optimization of the extraction step required careful consideration of sample pH. Acidic conditions promote the neutral, non-ionized form of the molecule, which presents greater lipophilicity and, consequently, higher partitioning efficiency into the organic extraction phase [2]. Working under neutral or alkaline pH conditions yielded no satisfactory recovery. Among the three organic solvents evaluated, ethyl acetate consistently outperformed dichloromethane, which produced only a faint, poorly defined spot, while diethyl ether failed to recover any detectable amount of pregabalin from the urine matrix. The superior extraction performance

of ethyl acetate under acidified conditions is consistent with the approach described by Golubev et al. and reflects pregabalin's hydrophilic character, which necessitates prior conversion to its less polar form to facilitate selective partitioning away from endogenous urinary components.

Mobile phase selection was driven by the polarity requirements imposed by pregabalin's physicochemical profile. Non-polar systems based on ethyl acetate and acetone failed to elute PGB, which remained immobile at the application point (Rf = 0). Polar systems containing methanol achieved satisfactory migration: pure methanol yielded an Rf of 0.42, while the methanol–ammonia mixture (98.5:1.5, v/v) shifted the Rf to 0.56 and, critically, resolved co-elution of furosemide and azithromycin that was observed under pure methanol alone, yielding cleaner chromatographic profiles with well-separated, reproducible spots. Examination of developed plates under UV irradiation at 254 nm and 366 nm revealed no visible spots for pregabalin, confirming the absence of significant UV absorbance at these wavelengths. Among the broad panel of colorimetric detection reagents screened, ninhydrin combined with thermal activation at 60°C was the sole reagent producing an unambiguous, clearly discernible violet spot. This response is rooted in the well-characterized reaction between ninhydrin and primary amino groups, yielding the purple condensation chromophore known as Ruhemann's complex [13, 14].

Method validation was conducted in accordance with established toxicological guidelines for qualitative screening methods [15]. Selectivity was assessed by demonstrating the complete absence of any interfering spot at Rf = 0.56 in six blank urine matrices sourced from different donors, each processed independently under identical conditions. Specificity was investigated by testing a panel of 30 substances encompassing commonly prescribed medications, psychotropic agents, and drugs of abuse, including gabapentin, methamphetamine, MDMA, cannabis, tramadol, codeine, buprenorphine, cocaine, fentanyl, olanzapine, levomepromazine, haloperidol, imipramine, lamotrigine, valproic acid, carbamazepine, ketamine, several benzodiazepines, anti-inflammatory agents, and antibiotics. No interference was observed, except for furosemide and azithromycin under the pure methanol system, which was effectively resolved by switching to the methanol–ammonia mobile phase. The limit of detection (LOD) was established at 100 µg/L, defined as the lowest concentration producing a distinct, reproducible violet spot in all six independent replicates. This sensitivity level is 5 to 10-fold below the cut-off values of commercially available immunoassay strip tests, substantially extending the diagnostic window of the method. Stability testing demonstrated that pregabalin remains reliably detectable in spiked urine stored at −20°C for seven days, while loss of detection occurred after three days of refrigerated storage at +4°C, indicating progressive compound degradation under these conditions. All validation results are summarized in Table 1.

Results

The method was applied to 87 urine samples collected from patients presenting to emergency departments of hospitals in the Central Region of Algeria and analyzed at the Toxicology Department of CHU Mohamed Lamine Debaghine, Algiers. Two groups were constituted: Group 1 comprised 77 samples with a positive immunoassay result (cut-off ≥ 500 µg/L), analyzed by TLC and GC-MS to assess concordance and investigate potential cross-reactivity; Group 2 comprised 10 samples with a negative immunoassay result despite clinical suspicion of pregabalin consumption, analyzed by both methods to evaluate TLC performance at sub-cutoff concentrations. In Group 1, TLC confirmed a positive result in 70 samples (91%), while the remaining 7 (9%) tested negative. GC-MS analysis of these 7 discordant samples identified gabapentin in all

Table 1. Method Validation Parameters.

Parameter	Result	Acceptance Criteria
Selectivity	No endogenous interference (6/6 samples)	No spots at Rf = 0.56 in blanks
Specificity	30/30 drugs showed no interference	Clear separation from interferents
Limit of Detection	6/6 at 100 µg/L	Visible spot after detection in all replicates (n=6)
Stability	Stable for 7 days at -20°C	Pregabalin still detected after 7 days at -20°C

Table 2. Clinical application of the TLC method to 87 urine samples from the Central Region of Algeria.

N° Group	Samples	Immunoassay	TLC	GC-MS	Interpretation
1	77	Positive (PGB)	70 Positive	Presence of PGB	True positive concordance
			7 Negative	Presence of Gabapentin, not PGB	False positive immunoassay cross-reactivity with gabapentin
2	10	Negative	Positive	Presence of PGB	False negative immunoassay TLC superior sensitivity

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cases and confirmed the complete absence of pregabalin, unequivocally attributing the false immunoassay positivity to gabapentin cross-reactivity — a clinically significant limitation that underscores the risk of misdiagnosis when relying solely on immunoassay results [8]. In Group 2, TLC detected a violet spot at Rf = 0.56 in all 10 samples, and GC-MS subsequently confirmed the presence of pregabalin at concentrations below the immunoassay cut-off in every case. These findings are detailed in Table 2.

Discussion

The superior analytical sensitivity of the TLC method (with an LOD of 100 µg/L compared to the immunoassay cut-off of ≥ 500 µg/L) carries direct clinical implications. It extends detection capability to scenarios in which pregabalin concentrations remain below the immunoassay threshold but are nonetheless toxicologically significant: first-time or intermittent use, early post-ingestion sampling, pediatric accidental poisoning, and in utero exposure in neonates born to drug-dependent mothers, for whom meconium analysis has recently been proposed as a complementary prenatal biomarker [9]. Simultaneously, the ability of TLC to distinguish pregabalin from gabapentin - a structural analogue not detectable by the pregabalin-specific ninhydrin reaction under the optimized chromatographic conditions - provides a straightforward means of resolving false-positive immunoassay results without recourse to GC-MS confirmation. Together, these properties position TLC as a meaningful, two-directional complement to immunoassay screening: correcting both false negatives arising from insufficient immunoassay sensitivity and false positives arising from cross-reactivity. The validated, chloroform-free TLC procedure described in this study offers a pragmatic, affordable, and analytically robust solution for qualitative urinary pregabalin detection, well suited to the operational realities of forensic and clinical toxicology laboratories in resource-limited settings. Its implementation may contribute to improved surveillance and case management in contexts where pregabalin misuse is escalating, and chromatography-mass spectrometry platforms remain inaccessible.

Conclusion

The validated, chloroform-free TLC procedure described in this study offers a pragmatic, affordable, and analytically robust solution for qualitative urinary pregabalin detection, well suited to the operational realities of forensic and clinical toxicology laboratories in resource-limited settings. Its implementation may contribute to improved surveillance and case management in contexts where pregabalin misuse is escalating, and chromatography-mass spectrometry platforms remain inaccessible.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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