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Original Research Article

Identification of Pregabalin in Human Urine Using Gas Chromatography Mass Spectrometry Method

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Abstract:

A rapid, simple method is presented for the identification and determination of pregabalin (PREG) in human urine by gas chromatography mass spectrometry (GC/MS) Solid phase extraction was performed using Strata TM-X-C33 µm polymeric strong cation columns (100 mg/3 mL tubes) to collect the extracts. The eluted extract was then derivatized directly in the column using N-methyl-N-(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (MSTFA). This method is specific for identifying PREG in urine, even when more than 10 different drugs of abuse are present. It easily detects both PREG-H2O MSTFA and PREG 2MSTFA, which have a relative retention time (RRt) of 0.9. At 1000 ng/ml, absolute recovery is 94.2%. Within-day precision (CV%, n=5) is 8.1% for PREG 250 and 0.3% for 1000 ng/ml. The method demonstrates linearity over 250-1000 ng/mL, with a limit of detection (LOD) of 250 ng/mL and a limit of quantitation (LOQ) of 500 ng/mL. These results show the method is highly suitable for routine identification of the drug in toxicology trace analysis.

Keywords: Pregabalin, Human Urine, GC/MS, SPE.

Introduction

Pregabalin (PREG), or (S)-3-(aminoethyl)-5-methylhexanoic acid, is a derivative of aminobutyric acid (see Fig. 1), with the chemical formula C8H17NO2 and molecular weight of 159.23 g/mol. It appears as a white crystalline powder, and is highly soluble in water as well as acidic and basic solutions [1,2].

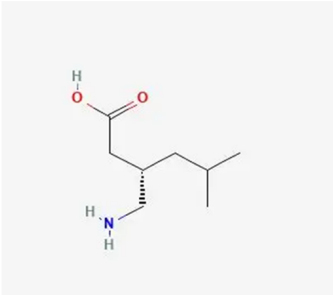

Figure 1 : Chemical Structure of Pregabalin

PREG received medical use authorization in the United States in 2004 [3] and was developed as a successor to gabapentin. It is available as a generic medication in several countries, though not in the United States as of 2028 [4]. Structurally, PREG is analogous to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and acts as a modulator of voltage-gated calcium channels in the central nervous system (CNS).

The molecule exerts its effects on voltage-gated calcium channels by binding presynaptically to the alpha-2-delta subunit. As voltage-gated calcium channels are widely distributed in the central nervous system, PREG can modulate the release of several excitatory neurotransmitters, including glutamate, substance P, norepinephrine, and calcitonin gene-related peptide.

This modulation inhibits overexcited neurons, ultimately restoring them to normal function. Additionally, PREG has a beneficial effect on sleep and sleep architecture, characterized by increased slow-wave sleep. PREG is mainly renally excreted, with 98% eliminated unchanged in the urine, and less than 0.1% through the fecal route. Based on

preclinical studies, PREG does not appear to undergo racemization to the R enantiomer in the body.

PREG is absorbed from the intestine by an active transport process mediated by the large neutral amino acid transporter (LAT1–SLC7A5), which also transports amino acids such as L-leucine and L-phenylalanine [9,10].

The objective of the present study was to identify pregabalin in human urine utilizing a rapid, robust, and validated gas chromatography-mass spectrometry method with an automated solid-phase extraction procedure.

Materials and Methods

Drug Standards: Pregabalin and Temazepam D5, drug standards from Serilliant Corporation – US.

Chemicals: Dichloromethane, Methanol, Ethyl acetate, 2-Propanol, and Ammonia solution from Merck KGaA.64271 Dormatadt -Germany; N – Methyl – N – (Trimethylsilyl)Trifluoroacetamide with 1% Trimethylchlorosilane from Sigma Aldrich Co. 3050Sprues Street, St. Louis, MO 63103 – USA (MSTFA); Deionized water obtained from Millipore devises Millipore Essential AFS.

Materials and Instruments: Gas chromatography mass spectrometry GC-MS QP2020 NX interfaced to AOC 20i Plus from Shimadzu; Rapid Trace (SPE) Zymark Consulting Part No 56846; Extraction column, strata – X -C33 µm polymeric strong cation, 100 mg/3 ml tube part No 8B-S029 EBJ, from Phenomenex.

Analytical Procedure: To 3 mL of urine spiked with PREG at concentrations of 125, 250, 500, or 1000 ng/mL, add 50 µL of a 10 µg/mL temazepam-D5 solution in methanol as the internal standard, followed by 500 µL of 10% phosphoric acid.

Centrifuge the sample for 5 minutes at 3000 rpm. Load the resulting supernatant onto the automated SPE module, following the reagent setup listed in Table 1 and using the PREG2 procedure described in Table 2.

The eluate was concentrated by evaporating to dryness in a water bath at 40ºC under a nitrogen stream. Residues were silylated by dissolving in a 100 µL mixture of 1:1 ethyl acetate and MSTFA. A 1 µL aliquot was injected into the GC/MS system, which was interfaced with an AOC. Injections occurred in splitless mode.

The initial column temperature was set at 70ºC and programmed to increase at 25ºC/min to a final temperature of 300ºC, which was held for 5 minutes. Injection and interface temperatures were adjusted to 250ºC and 280ºC, respectively. Helium served as the carrier gas at a flow rate of 20 cm/s. Electron impact (EI) ionization was performed, obtaining spectra at 70 eV, scanned from 50–400 a.m.u. at a rate of 1.88 a.m.u./ms. The source temperature was maintained at 180ºC.

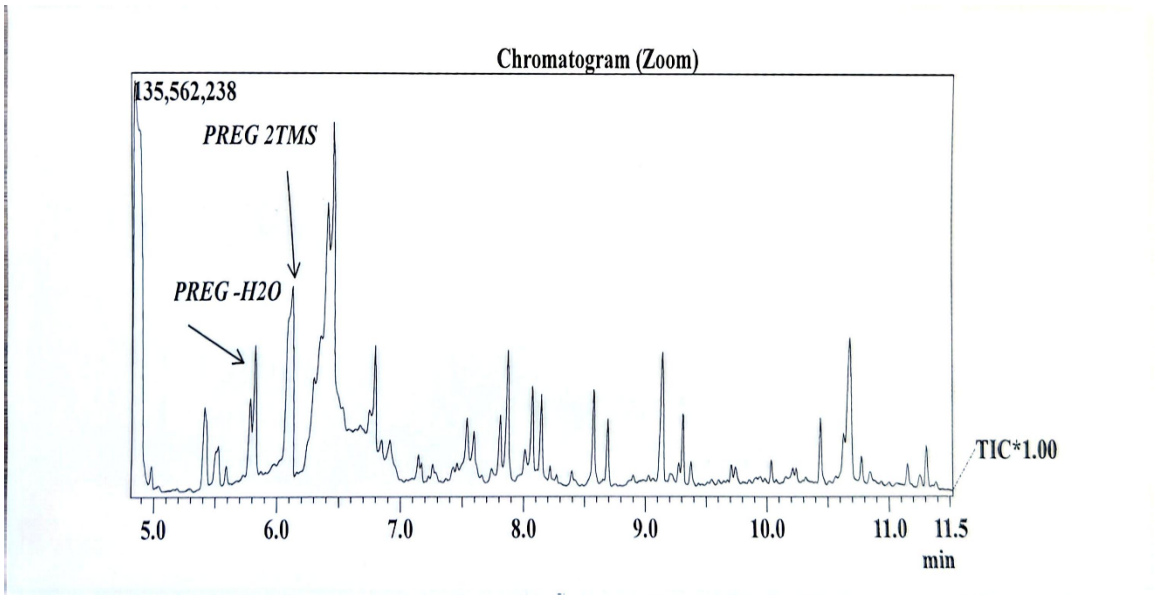

Figure 2: Reconstructed Ion chromatogram, full Scan (TIC) of Urine PREG sample collected from suspected person after oral administration.

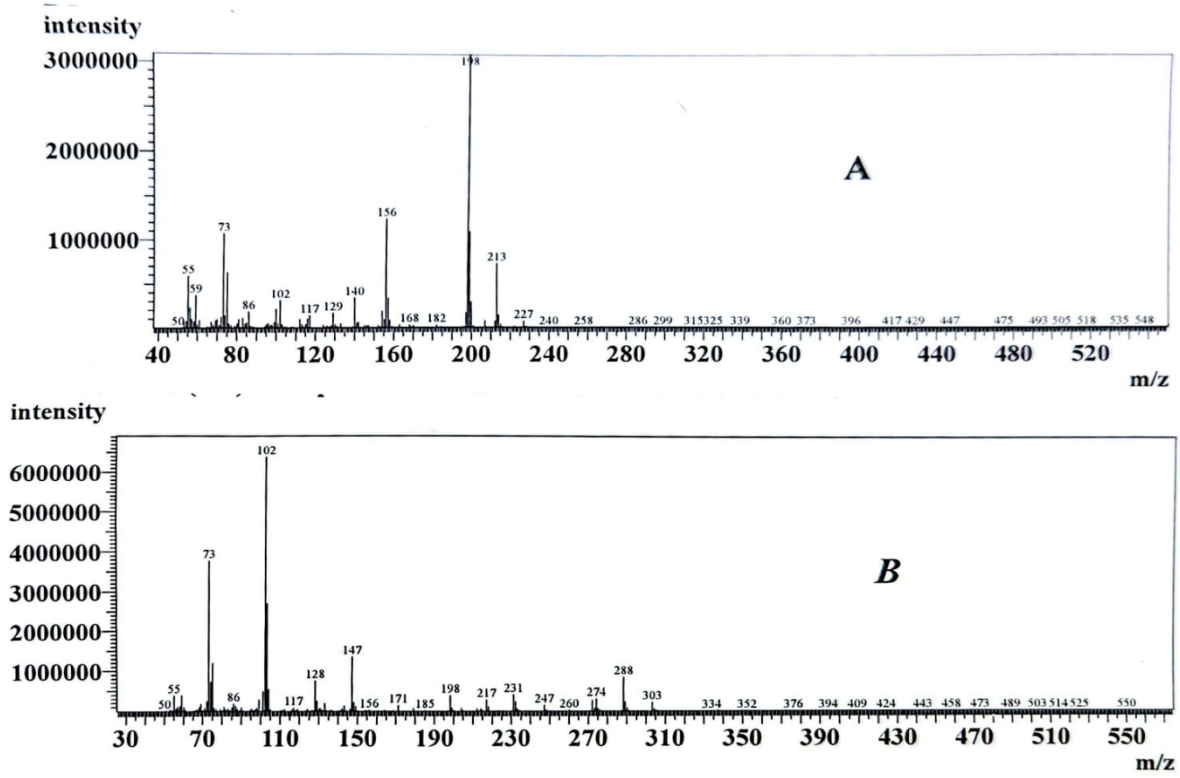

Figure 3: Positive EI Spectrum of PREG -H2O -MSTFA (A) and PREG 2TMS (B) Isolated from Positive Urine Sample Collected after oral Administration of PREG.

Table 1: Reagents setup for Pregabalin SPE

Reagent Setup	Reagent Name	Abbreviation	Sip Speed
1	4% Phosphoric Acid	4% Phosph	30
2	Methanol: Water 50:50	50:50	30
3	Dichloromethane: Isopropanol: Ammonia 78:20:2	Elute	15
4	Methanol	METH	30
5	De-ionized Water	H2O	30

Table 2: Pregabalin SPE Procedure

No	Step	Source	output	volume	mL/min	Liquid sense
1	Load	Sample	AQU	4 ml	1.2	NO
2	Purge Cannula	H2O	Cannula	4 ml	30	NO
3	Rinse	4% phosph	ORG	3 ml	12	NO
4	Rinse	4% phosph	ORG	3 ml	12	NO
5	Dry		time	0.6	min	NO
6	Rinse	50:50	ORG	3 ml	12	NO
7	Rinse	50:50	ORG	3 ml	12	NO
8	Dry		time	10	min	NO
9	Collect	Elute	Fract1	3 ml	1.2	NO
10	Purge Cannnula	Meth	cannula	3 ml	30	NO
11	Purge Cannnula	H2O	cannula	3 ml	30	NO

Table 3: Summary of accuracy, precision, and recovery results of proposed method

Proposed method	Concentration µg/ml			CV %	% Recovery
	Taken µg/ml	n	SD		
Intra - day assay	0.250	5	1.01	2.7	70
	0.5	5	2.2	7.5	
	1	5	1.01	3.1	94.2
Inter - day assay	0.5	5	2.0	5.9	

Results and Discussion

In this method, chromatographic analysis of urine samples positive for PREG after oral administration showed retention times of 5.8 minutes for hydrated PREG and 6.2 minutes for PREG 2TMS, as illustrated in Figure 2. Hydrated PREG-MSTFA produced ion fragments at m/z 198 (base ion) and m/z 213 (molecular ion) (Figure 3A), while PREG 2TMS produced a base ion at m/z 102 and a molecular ion at m/z 303 (Figure 3B). The relative retention time (RRt) of hydrated PREG compared to PREG was 0.9, providing reliable evidence for drug identification and confirmation.

Within-day precision assays were performed by analyzing PREG at concentrations of 0.250, 0.5, and 1 µg/mL (n=5). For interday precision, urine spiked with the drug was analyzed on consecutive days for one week at 0.5 µg/mL. These results are summarized in Table 3. At 0.5 µg/mL, the signal-to-noise ratio was 371.08. The method's selectivity was evaluated by testing PREG-positive samples in the presence of drugs such as gabapentin, tramadol, alprazolam, amphetamine, paracetamol, codeine, and typical urine impurities; none interfered with the assay. The method afforded recoveries of 94.2% at 1 µg/mL and 70% at 0.250 µg/mL, as determined from a one-point calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were established by analyzing PREG at 0.5, 0.250, and 0.125 µg/mL (n=10); the signal-to-noise ratios were 156.1 for 0.250 µg/mL and 371.08 for 0.5 µg/mL, indicating LOD and LOQ of 0.250 and 0.5 µg/mL, respectively. The described GC/MS method has been demonstrated to be both accurate and sensitive for detecting pregabalin in human urine [references 11, 12, 13].

Conclusion:

The proposed method does not require any laborious cleanup procedure before measurement. In addition, the method has a wider linear dynamic range with good accuracy and precision. The method shows no interference from the common urine impurities and drug abuse taken with the same drug. The method has been in use for routine identification of the drug for more than 1 year in our laboratory without any problems. The LOD of the method is 250 ng/ml. Demonstrate that it is easy and efficient to determine the amount of 500 ng/mL, the cut-off for the drug in urine in the United Arab Emirates (UAE). The experimental results for linearity, accuracy, precision, specificity, and sensitivity demonstrate the method's reliability for its intended application.

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