Journal Of Advanced Pharmaceutical Sciences
Journal homepage: https://japs.journals.ekb.eg/

Original article

# Qualitative and quantitative analysis of some tramadol registered and illegitimate dosage forms in Egypt

**Hebat-ALLAH I. Mandour*[a,b], Tarek S. Belal[c], Alyaa A. Ramadan[a], Fouad M. Sharabi[d], Mohamed A. Shamseddeen[a]**

[a] Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
[b] Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
[c] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
***Corresponding author:** Faculty of Pharmacy, Alexandria University, 1 Khartoum Square, Azarita, Messalla Post Office, P.O. Box 21521, Alexandria, Egypt
E-mail: hebatallah.mandour@alexu.edu.eg

**Abstract:**


Despite tramadol being a strong pain relief authorized medication, it is currently considered among the most highly abused substances in Egypt. Due to consequent strict legal acts on the registered brands of tramadol, available illegitimate products were widely distributed on the streets in Egypt. The claim of many patients receiving treatment while suffering from seizures together with the assumption of inferior authenticity of these products led the initiative to work on the qualitative and quantitative analysis of such products while comparing their content to the registered brands in the Egyptian market. Results obtained by applying a validated HPLC-DAD method as a highly efficient technique in pharmaceutical analysis revealed that most of these products' content was within the limit of the labeled claim except for one compound that showed an additional different peak.

Efforts to identify such peak through analysis and comparison of the spectrum referred to paracetamol, the well-known analgesic commonly combined with tramadol in its dosage forms. The employed HPLC procedure involved the use of RP-C18 column, mobile phase of phosphate buffer (pH 6.0), and methanol (40:60, v/v) and diode array detection at 220 and 270 nm. Accurate, precise, and reliable testing procedures under governmental surveillance are required to assure persistent quality, and safety of the available drugs on the market.

**Keywords: Tramadol use, illegitimate products, counterfeit, HPLC.**

Received 13 December 2023 Accepted 27 January 2024 Published 28 January 2024



DOI 10.21608/JAPS.2024.255337.1011
ISSN: 3009-7061

## 1. Introduction

In the pharmaceutical formulation industry, the final products undergo several processes of quality testing to ensure that the quality, safety, and effectiveness of their active pharmaceutical ingredients are well maintained before their release into the market. This quality assurance can be achieved via a variety of analytical techniques that provide critical data about the identity, purity, strength, potency, and content of the active pharmaceutical ingredients (API). New innovative methods have been developed by different organizations and manufacturers to improve the health-related quality of mankind. This is all brought into worldwide harmonization through the regulations of the International Council for Harmonization (ICH). Quantitative tests for API characterization are among the fundamental tests required to describe the various aspects of a pharmaceutical product, together with the compendia tests mentioned in the pharmacopeia as well as other tests that are concerned with solid-state characterization [(1)].

Uniformity of content of solid dosage form preparations is an essential test among the implemented pharmacopeial specifications and international standards required to maintain the pharmaceutical quality of single-dose formulations. These requirements are fundamental to put limits for permissible variations of the quantity of API within the individual single-dose units to assure that any patient would receive the API in an amount as close as possible to what is claimed on the label of the pharmaceutical product [(2)].

Upon aggravation of the problem of wide distribution of counterfeit, spurious, and illegal drugs, the urge for analytical techniques became crucial for the detection and quantification of API within these products. Pharmaceutical counterfeiting, which is usually of Asian origin, includes products that may either contain no API at all, an insufficient amount of the API, another different API, or maybe with fraudulent packaging. Hence the need for analytical laboratory approaches was evident for the assessment of further undesirable public health risks and to provide a basis for decision makers in health authorities. Chromatographic approaches showed higher advantages over the commonly applied spectroscopic methods. They are not only considered as detection and quantification tools but moreover, they support separation of the components therefore they present a whole picture for the composition of the analyzed products. This property of product characterization would be of important value regarding what illicit manufacturers put in order to mask the detection of synthetic compounds by spectroscopy [(3,4).]

High-Performance Liquid Chromatography (HPLC) represents a reliable standard method that is highly applied in industry for quantitative analysis of various pharmaceutical products with remarkable reproducibility. UV/ visible detection monitoring when combined with HPLC assures more accuracy, precision, and robustness of the applied methods. The diode array detector (DAD) provides a more sophisticated tool for the determination of the purity of peaks eluting from HPLC columns. The variable choice of HPLC column chemistry, the variety of attached detectors, and the possibility of performing the analysis on a fully automated instrument make the HPLC the most suitable analytical technique for the quantitative determination of a wide range of API with their related substances in the same run [(5,6)].

Tramadol HCl (TH) is an official drug at the British, European, and the United States Pharmacopeia [(7)]. The USP suggests HPLC among the methods applied for the assay of the powder and as the main method for the



ISSN: 3009-7061

assay of tablets. The British Pharmacopeia suggests HPLC for the assay of capsules [8]. In literature, several spectrophotometric and chromatographic techniques were applied for its determination and analysis in various dosage forms whether it is alone or combined with other drugs as well as being analyzed in its powder form and for its quantification in plasma or biological fluids [7-15]. Various analytical techniques have been reported for either quantification of tramadol individually or its simultaneous quantification if combined with other drugs, especially analgesics such as paracetamol [8,9,11,13], aceclofenac [9], metamizole, ropivacaine, bupivacaine [15], diclofenac sodium or muscle relaxants such as chlorzoxazone [7].

The effect that is desired to be obtained from the use of tramadol sometimes depends on the compounds or medications that are taken with it. Among these medications is paracetamol, which is frequently administered in fixed-dose combinations with tramadol to provide multimodal analgesia for patients with moderate to severe pain [16]. Whereas sildenafil with its known effect in the treatment of erectile dysfunction can be concomitantly administered with tramadol for its role in the treatment of premature ejaculation. Their combined effects are the reason beyond the recreational use of such combinations [17]. On the other hand, diazepam can be used with tramadol for the benzodiazepine protective effect against the severity of seizures that may occur as a side effect of tramadol use [18]. The risk of development of seizures is higher with tramadol use than with opioids [19, 20]. These tramadol-induced seizures are more likely to be dose-dependent, yet they can occur in some cases with the recommended therapeutic doses [21, 22]. Moreover, concomitant administration of antidepressants or neuroleptics, or other drugs that reduce the seizure threshold may potentiate the seizure-producing effects of tramadol [23].

Presumably, drugs that are formulated for street sale are typically doubted to be impure and consist of a mixture of psychoactive substances that may produce toxic reactions when combined or to be of reduced content variability and/or lower manufacturing quality. However, the users of illegally distributed drugs are mostly unaware of the actual included chemical substances, the dose being purchased, and the contaminants that may exist in the drugs they take [24]. According to the United Nations Office on Drugs and Crime (UNODC), World Drug Report, 2020, there is still little or no available information on the nature and actual content of the psychoactive substances that may be contained in many of the largely distributed new illicit drugs [25].

Regarding the situation in Egypt, the epidemic spread of tramadol that occurred in the previous years can be attributed to variable factors based on its illegal availability on streets, the misconception about its effects on increasing sexual potency, ability to concentrate, and maintaining endurance against workloads in addition to the convenience of its use orally rather than the need for injections [26]. Addicts of tramadol specifically suffer from seizure attacks that may be severe enough to make them faint [27].

Egyptian therapists used to assume that illegitimate abused dosage forms must contain certain adulterating compounds responsible for such annoying manifestations. Accordingly, this part of the study was intended to conduct a qualitative and quantitative analysis of some selected products that contain tramadol as their main active ingredient through the application of chromatographic approaches. The products tested included those obtained from legal authorities within the procurement distribution cycle of drugs in the Egyptian



ISSN: 3009-7061

market, as well as the products illegally distributed on local streets. The chosen dosage forms were analyzed to:

- Ascertain that these illegal dosage forms abused by a big percentage of the public do contain TH as their main active pharmaceutical ingredient (API) as their labels claim.
- Determine the variation in the content of TH in these selected pharmaceutical products to test whether the illegitimate products may contain more or less than the claimed concentration on the labels of their packs.
- Investigate the possible presence of adulterants in the selected products to quest the assumption of seizures occurrence due to the existence of other substances in the pharmaceutical formulation than the active ingredient.

To the far best of our knowledge, we believe that this directed work for adulterants identification in illegitimate widely distributed dosage forms of tramadol addresses an important problem and represents a good contribution to the field of pharmacy practice where potential future research can proceed in treatment and prevention of addiction.

## 2. Materials and Methods

### 2.1. Instrumentation

The HPLC-DAD system consisted of Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, USA) (quaternary pump, vacuum degasser, and diode array and multiple wavelength detector G1315 C/D and G1365 C/D) with standard auto-sampler, connected to a computer loaded with ChemStation Explorer Software. Effective chromatographic separation was achieved using Equisil BDS C18 column, 5 µm particle size (250×4.6 mm i.d.). A nylon syringe filter of 0.45µm pore size was used for the filtration of solutions before injection into the column.

### 2.2. Materials

#### 2.2.1. Standards

Standard TH was supplied by Fluka BioChemika, Buchs, Switzerland. Other standards used in the study include paracetamol, diazepam, and sildenafil.

#### 2.2.2. Reagents and solvents

Methanol (HPLC-grade, Avantor Performance Materials, Gliwice, Poland), Sodium dihydrogen orthophosphate dihydrate (Analar, BDH Chemicals Ltd., Poole, England), analytical reagent-grade Anhydrous disodium hydrogen phosphate (El Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt) and high purity deionized water.

#### 2.2.3. Samples

The pharmaceutical dosage forms assayed in this study include two Egyptian registered brands of tramadol that were legally distributed on the Egyptian market at the time of conducting the study: Tramundin® retard tablets; sample A (MUP; Medical Union Pharmaceuticals, Ismailia, Egypt, under license by Mundipharma, Basel, Switzerland) labeled to contain 100 mg TH per tablet and was supplied with appreciation by the authority of the pharmacy in the Medical Research Institute, Alexandria University, and Contramal® capsules; sample B, kindly donated by the manufacturing company (SIGMA Pharmaceutical Industries, Egypt) labeled to contain 50 mg TH per capsule.

The assayed illegitimate products of tramadol that were confidentially provided under strict surveillance include Tramajack® hard gelatin capsules; sample C, labeled to contain 125mg TH (with no assigned manufacturer on the label), Tramadol-X 225® red film coated tablets; sample D (Raya, India) labeled to contain 225 mg TH, Tamol-X® white coated engraved tablets; sample E (assumed manufacturer as Royal, USA) labeled to contain 225 mg TH, Tamol-X® white coated un-carved tablets labeled to contain 225 mg



ISSN: 3009-7061

TH per tablet; sample F (with no assigned manufacturer on the label), Tamol-X® white coated un-carved tablets, labeled to contain 200mg TH; sample G (with no assigned manufacturer on label) (**Table 1**).

**Table 1: TH-containing dosage forms under study.**

| Sample | Trade name and Dosage form | Labeled TH amount/tablet or capsule in mg |
|---|---|---|
| **A** | Tramundin® retard tablets | 100 |
| **B** | Contramal® hard gelatin capsules | 50 |
| **C** | Tramajack® hard gelatin capsules | 125 |
| **D** | Tramadol-X® red film-coated tablets | 225 |
| **E** | Tamol-X® white coated engraved tablets | 225 |
| **F** | Tamol-X® white coated un-carved tablets | 225 |
| **G** | Tamol-X® white coated un-carved tablets | 200 |

## 2.3. Methods

### 2.3.1. Chromatographic conditions:

This work depends on the application of the already reported method for the HPLC analysis of tramadol in pharmaceutical dosage forms [(9)]. Method and chromatographic conditions are adopted in this study. Phosphate buffer (pH 6.0) was prepared by dissolving 0.27 g anhydrous disodium hydrogen phosphate and 1.56 g sodium dihydrogen ortho-phosphate dihydrate in 500 mL of freshly prepared high-purity deionized water. Components were mixed, Triethylamine (1 mL) was added to improve peak shape and reduce tailing [(9)], sonication for 10 minutes then completing the volume till 1000 mL. The mixture was then adjusted to reach pH 6.0 by the addition of a few drops of ortho-phosphoric acid. The prepared buffer was then filtered using a cellulose membrane filter of 0.45µm. The mobile phase is composed of the prepared phosphate buffer (pH 6.0) and methanol in the mixed proportion of 40:60 (v/v) respectively. The flow rate was set to be 1.0 mL /minute, with an injection volume of 20 µL. The diode array detection was carried out at λ = 220 nm and 270 nm. All measurements were performed at room temperature. Aliquots of the mobile phase were used to dilute the working solutions of the standard and the sample.

### 2.3.2. Standard solutions:

Stock solutions of TH, paracetamol, diazepam, and sildenafil (1000 µg/mL) were separately prepared in methanol.

### 2.3.3.Working standard solutions.

Aliquots of the stock standard solution were diluted using the mobile phase using a vortex stirrer to prepare the working solutions of the standard of concentration range 50-300 µg/mL. Injections were made for each concentration, filtered via a nylon syringe filter (0.45 µg pore size), and analyzed under the previously described chromatographic conditions.

### 2.3.4. Determination of regression equation.

Each concentration of the range 50-300 µg/mL was made into triplet injections, each of 20 µL, that were chromatographed under the previously described chromatographic conditions. For each concentration, the peak areas at the specified wavelengths were determined, and linear regression equations were calculated.

## 2.4. Assay of the marketed pharmaceutical dosage forms

### 2.4.1. Preparation of the tablet solutions

Ten tablets of each of the tested TH-containing dosage forms were crushed and finely powdered. An accurately weighed amount equivalent to 100 mg of TH was transferred to a 100 mL volumetric flask, extracted with 50 mL of methanol, sonicated for 10 minutes for complete dissolution, and then the volume was made to the mark with methanol to make a concentration of 1000 µg/mL (1 mg/mL). The same procedure is



ISSN: 3009-7061

repeated for all analyzed tablet dosage forms. Capsules were emptied and their powder content was treated the same as the crushed tablets. From the above solution, 1 mL was transferred to a 10 mL volumetric flask and diluted to volume with the mobile phase using vortex to ensure complete mixing. The prepared sample solutions were then filtered by a 0.45 µm Nylon syringe filter and then analyzed using the previously described chromatographic conditions.

## 3. Results and discussion

### 3.1. Selection of chromatographic conditions

Samples (A-G) were successfully analyzed by the previously described adopted HPLC method [(9)]. TH showed maximum absorbance at 220 nm and 270 nm. Chromatograms obtained at 270 nm were selected where parameters such as good peak areas, acceptable retention time ($t_R$), acceptable asymmetry, number of theoretical plates, and good resolution were obtained. The average retention time ($t_R$) for TH was found to be 6.44 ± 0.042 minutes.

### 3.2. Validation

The applied adopted method was previously validated in terms of linearity, range, precision, and accuracy as indicated in the relevant publication [(9)].

The regression equation for the analysis of TH using the adopted HPLC-DAD method was: A = - 28.243 + 6.590 C, with a correlation coefficient of (r = 0.99991), where A is the peak area at 270 nm and C is the concentration of the drug in µg/mL. Regarding the quantitative analysis and the regression equation, adherence of the system to linearity was validated by the high value of the correlation coefficient.

### 3.3. Assay results

#### 3.3.1. Quantitative analysis of the pharmaceutical dosage forms

Regarding the quantitative determination of the content of TH in the analyzed dosage forms, **Table 2** summarizes the recovery % ± standard deviation of five replicate determinations for each selected product of TH. Results indicated that the content of the active ingredient recovered on the various dosage forms analyzed in this study were within an acceptable limit as in agreement with the label claim. Surprisingly, findings showed that the highest percentage of recovery is attained with an illegitimate product (sample E) while sample C was the product with the lowest percentage of recovery. In general, the calculated recovery % of the analyzed tablets was NLT 90.0% and NMT 110.0% of the labeled amount of TH, which was consistence with what is mentioned in USP 38 [(28)]. While according to the BP, the allowed content of TH in capsules, ranges from 95.0% to 105.0% of the stated amount [(29)], which did not coincide with the obtained results of sample C. Moreover, the low values of standard deviation are indicative that the adopted chromatographic method for the determination of tramadol in its dosage forms is accurate and precise.

**Table 2:** Analysis of some Tramadol dosage forms using the applied HPLC-DAD method.

| Sample | Mean recovery ± SD |
|---|---|
| **A** | 99.051 ± 0.998 |
| **B** | 93.707 ± 1.989 |
| **C** | 79.401 ± 1.784 |
| **D** | 96.907 ± 1.730 |
| **E** | 99.068 ± 1.835 |
| **F** | 94.168 ± 1.188 |
| **G** | 94.294 ± 0.238 |

*****Mean recovery ± Standard Deviation for five determinations.

Every chromatogram of the analyzed pharmaceutical dosage forms matched with the chromatogram of standard TH with identical values for the retention time (**Fig. 1, 2**). The spectra of all peaks that appeared in



ISSN: 3009-7061

chromatograms matched with the DAD-saved UV spectrum of TH. There were no interfering unidentified peaks within the selected detection wavelength in the HPLC chromatogram of most of the analyzed dosage forms except for the chromatogram of the illegitimate product (sample G) where it showed an additional peak with a retention time ($t_R$ = 3.037) earlier than that of tramadol (**Fig. 3**). The chromatogram of such a peak matched with the chromatogram of standard paracetamol which eluted at the same retention time ($t_R$ = 3.025). Moreover, the peak UV spectrum of sample G matched with the DAD saved spectrum of standard paracetamol (**Fig. 4-6**).

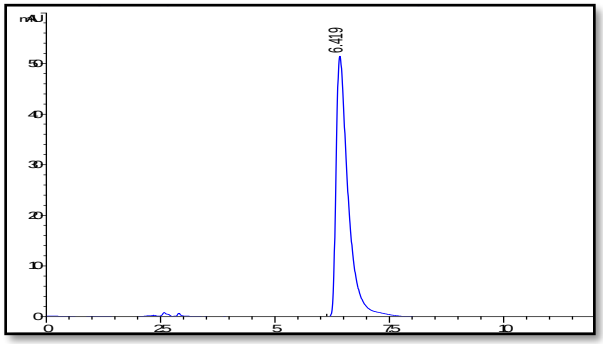


**Fig. 1: Representative HPLC-DAD chromatogram of sample A of 150 µg/mL TH, at 270 nm**

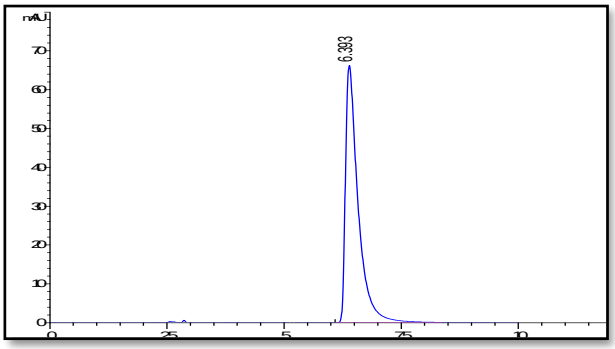


**Fig. 2: Representative HPLC-DAD chromatogram of sample C of 125 µg/mL TH at 270 nm**

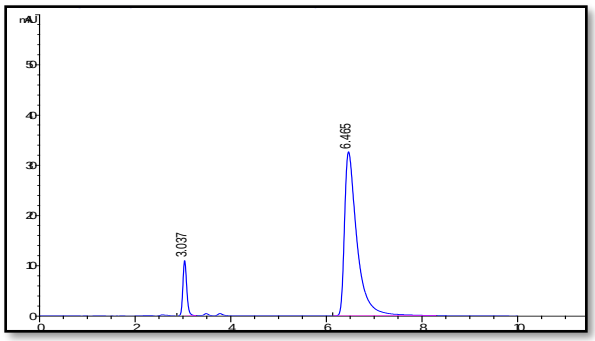


**Fig. 3: Representative HPLC-DAD chromatogram of sample G of 100 µg/mL TH at 270 nm.**

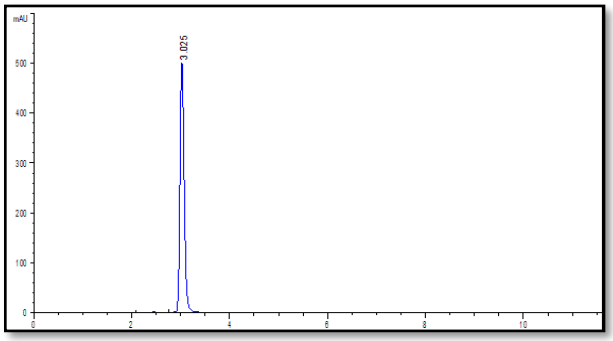


**Fig. 4: HPLC-DAD chromatogram of standard paracetamol of 1000 µg/mL at 270 nm.**

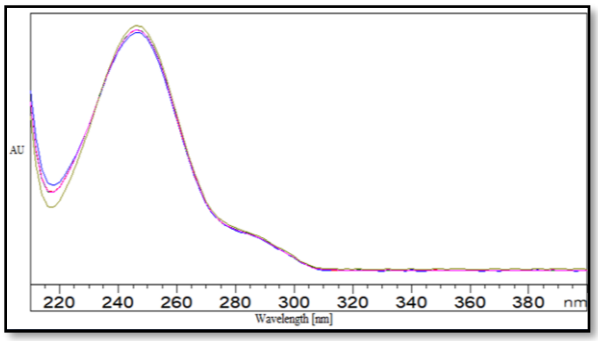


**Fig. 5: UV spectrum of unidentified peak eluting at $t_R$ = 3.037 min, obtained from DAD**

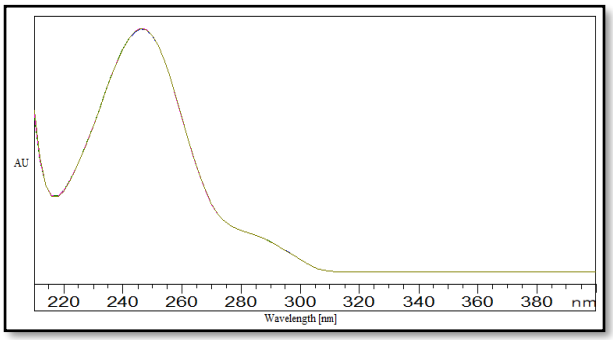


**Fig. 6: UV spectrum of standard paracetamol (1000 µg/mL), obtained from DAD**



ISSN: 3009-7061

### 3.3.2. Qualitative analysis of the pharmaceutical dosage forms

Regarding the qualitative analysis of the legally marketed and illegitimate pharmaceutical dosage forms of tramadol; applying the adopted HPLC-DAD chromatographic method made it possible to confirm the identity and assay tramadol in its available selected dosage forms.

The Diode Array saved spectrum of tramadol was used for the identification of TH in the analyzed illegitimate products. All the analyzed dosage forms showed the same chromatogram characteristics when compared to that of tramadol. This confirms the existence of tramadol as the main active ingredient of the analyzed dosage forms. The exception of one dosage form (sample G) that showed an additional peak for a compound eluted with a retention time earlier than that of tramadol ($t_R$ = 3.037), raised the suspicion of possible adulteration.

### 3.3.3. Identification of foreign peak

Upon attempts to identify the different peaks observed in the chromatogram of tramadol obtained from sample G, several standard compounds were proposed to be tested in order to identify this unknown peak and chromatographed under the same conditions. The tested compounds included sildenafil, diazepam, and paracetamol. The choice of analyzing these particular drugs was based on the suggestion of how far they may add to the addicts' favorable effects of tramadol if they were combined with it in the same pharmaceutical formulation. Tramadol is known for its effect on reducing premature ejaculation but still it may weaken the erection, so when administered concomitantly with PDEIs such as sildenafil, usually indicated for erectile dysfunction, they concomitantly may affect the ejaculation response time [30, 31]. And since this is considered one of the addicts' desirable effects of tramadol, the decision to analyze sildenafil was taken. Similarly, the choice of diazepam analysis followed the presumption of many therapists, that it could be combined in tramadol-containing dosage forms as being a medication of the benzodiazepine family commonly abused for its calming and seizure-risk-reducing effects. Such therapists' opinions were greatly considered as they work at Al-Maa'moura Hospital, which is located in Alexandria and is considered one of the biggest and most important hospitals in Egypt that specializes in the treatment of neuropsychiatric disorders with a distinct department for the management of addiction disorders under the supervision of the Egyptian Ministry of Health and Population. As for choosing paracetamol, it was moreover due to its frequent incorporation in many pharmaceutical dosage forms with tramadol that are analyzed in many research articles by several chromatographic techniques [8, 9].

As detected, results revealed that this peak cannot be referred to either sildenafil or diazepam due to differences in UV spectrum characteristics where diazepam and sildenafil eluted at $t_R$ = 8.800 and 13.762 minutes, respectively (**Fig. 7-10**). On the other hand, the chromatogram of the additional peak corresponded with that of standard paracetamol where both eluted approximately at the same retention time ($t_R$ = 3.03 min). Furthermore, the resemblance of the DAD saved spectrum of paracetamol and that obtained for the unidentified peak in sample G confirmed the identity of such peak, proving the presumption of paracetamol addition for an added synergistic analgesic effect.

The fact that results showed that all the analyzed samples contained the correct active



ISSN: 3009-7061

ingredient with mostly acceptable recovery of the drug (mean % recovery with low SD) and lack of interference of other ingredients in the selected pharmaceutical formulations, reduces the suspicion of counterfeiting. Results failed to support the view that the street-available dosage forms of tramadol have different or variable content than the label claim.

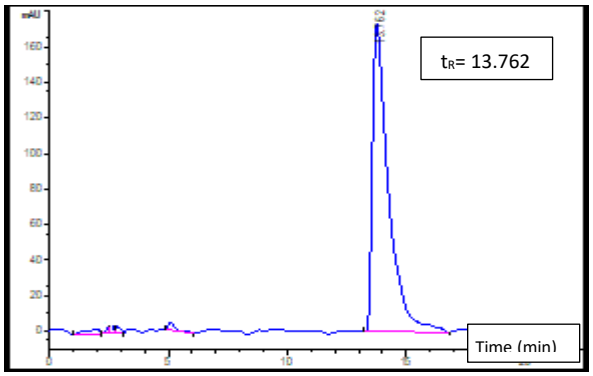


**Fig. 7: HPLC-DAD chromatogram of standard sildenafil (1000 µg/mL)**

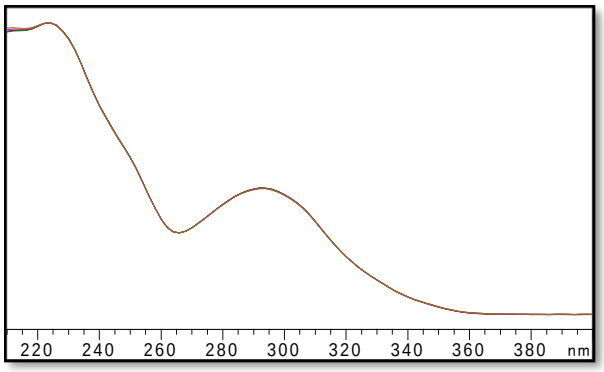


**Fig. 8: UV spectrum of standard sildenafil (1000 µg/mL) obtained from DAD**

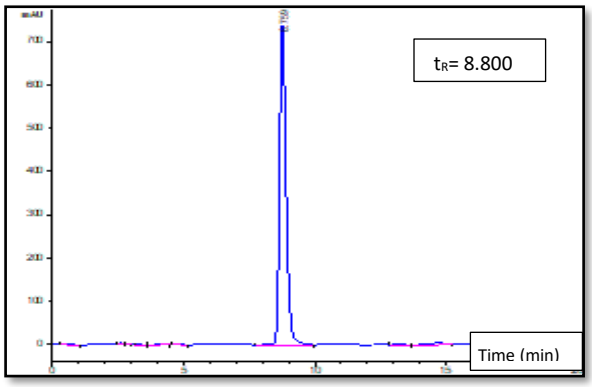


**Fig. 9: HPLC-DAD chromatogram of standard diazepam of (1000 µg/mL)**

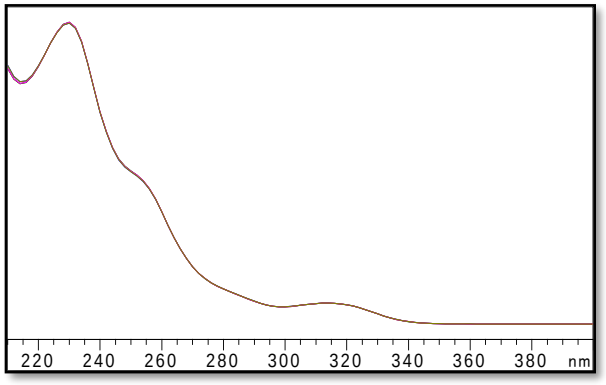


**Fig. 10: UV spectrum of standard diazepam of (1000 µg/mL) obtained from DAD**

## 4. Conclusions

HPLC is constantly a reliable method for the qualitative and quantitative determination of various active ingredients in their powder form or pharmaceutical formulations as well as for tramadol in its pharmaceutical dosage forms.

Satisfactory results are obtained, in high agreement with the label claims, for the analysis of different registered and illegitimate pharmaceutical products that contain tramadol. This is indicative of negative interference from any of the excipients that may be conventionally present in typical pharmaceutical formulations. Accordingly, and based on the obtained results, the assumption of possible adulteration or coexistence of other addictive substances or neurologically acting drugs in the same dosage form with Tramadol can be rejected. Despite their bad reputation and great doubts about their quality and safety, most of the analyzed illegally distributed TH-containing products showed acceptable recovery of their content.

Convulsions manifested upon prolonged abuse of higher daily doses of different Tramadol illegitimate products can be most



ISSN: 3009-7061

likely attributed to tramadol itself. This can be explained either by the side effects of tramadol intake in amounts far beyond the recommended therapeutic doses or its possible co-ingestion with other seizure threshold-lowering drugs.

Future research employing other analytical techniques and investigating approaches would be highly recommended to provide more definitive evidence and consolidate the existing findings.

A properly executed, governmentally funded monitoring approach that maintains continuous periodical qualitative and quantitative analysis for the variably distributed illicit substances would be highly recommended using more specific and sensitive instrumentations. This can be achieved under the surveillance of higher authorities in order to provide full documentation about the compliance of the pharmaceutical quality of these products for the aim of reducing further undesirable health risks among addicts.

**Conflict of interest**

The authors have declared no conflict of interest.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Data availability**

Data will be made available on request.

**Highlights**

- An HPLC-DAD adopted method was successful for the analysis of Tramadol-registered and illegitimate dosage forms in Egypt.
- Most analyzed tramadol illegitimate dosage forms showed acceptable content recovery.
- Only one extra peak for co-formulated paracetamol in a single tramadol illegitimate dosage form was identified within the selected detection wavelength.
- Seizures occur with tramadol use in doses higher than therapeutically recommended and not as assumed due to other concomitantly added adulterants.
- Pharmacists play an integral part in the surveillance of illegitimate dosage forms to protect society.

## 5. References

(1) Richardson CF. Compendial Testing. In: Handbook of Modern Pharmaceutical Analysis (Ahuja S, Scypinski S. eds.) Academic Press, USA; 2001 Jul 26:325-44.

(2) Martins JM, Farinha A. Uniformity of dosage units: comparative study of methods and specifications between Eur. Pharm. 3rd and USP 23. Journal of pharmaceutical and biomedical analysis. 1998 Dec 1;18(4-5):487-95.

(3) Deisingh AK. Pharmaceutical counterfeiting. Analyst. 2005; 130(3):271-9.

(4) Deconinck E, Sacré PY, Courselle P, De Beer JO. Chromatography in the detection and characterization of illegal pharmaceutical preparations. Journal of chromatographic science. 2013 Sep 1;51(8):791-806.

(5) Watson DG. Pharmaceutical Analysis: A textbook for pharmacy students and pharmaceutical chemists, 3rd ed. Churchill Livingstone, UK; 2012:440pp.

(6) Rasmussen HT. Method Development. In: Handbook of Modern Pharmaceutical Analysis (Ahuja S, Scypinski S. eds.) Academic Press, USA; 2001 Jul 26:345-86.

(7) Patel J, Patel P. RP-HPLC method development and validation for the estimation of diclofenac sodium, tramadol hydrochloride and chlorzoxazone from their combined tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(7):632-7.

(8) Belal T, Awad T, Clark R. Determination of paracetamol and tramadol hydrochloride in pharmaceutical mixture using HPLC and GC-MS. Journal of chromatographic science. 2009 Nov 1; 47(10):849-54.

(9) Chandra P, Rathore AS, Lohidasan S, Mahadik KR. Application of HPLC for the simultaneous determination of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form. Scientia pharmaceutica. 2012 Jun; 80(2):337-51.

(10) Desireddy RB, Kumar PJ, Chand AS, *et al.* Development and validation of RP-HPLC method for quantitative analysis of tramadol in pure and pharmaceutical formulations. International Journal of Chemical Sciences. 2012; 10(4):2039-47.

(11) Gharge D, Dhabale P. Simultaneous estimation of tramadol hydrochloride and paracetamol by



ISSN: 3009-7061

UV spectrophotometric method from tablet formulation. International Journal of PharmTech Research. 2010;2(2):1119-23.
(12) Kambl RM, Singh SG. Stability-indicating RP-HPLC method for analysis of paracetamol and tramadol in a pharmaceutical dosage form. E-Journal of Chemistry. 2012 Jan 1;9(3):1347-56.
(13) Shukla R, Shivkumar R, Shivan KN. Development of a UV-spectrophotometric method for the simultaneous determination of tramadol hydrochloride and paracetamol in bulk and marketed product. Bulletin of Pharmaceutical Research. 2011;1(1):62-6.
(14) Setty KN, Ramachar T, Chakravarthy IE, Prabhavathi K. A simple spectrophotometric estimation of tramadol hydrochloride in pharmaceutical formulations. Chemical Science Transactions. 2012;1(2),317-20.
(15) Salmerón-García A, Navas N, Martín A, Román E, *et al*. Determination of tramadol, metamizole, ropivacaine, and bupivacaine in analgesic mixture samples by HPLC with DAD detection. Journal of chromatographic science. 2009 Mar 1;47(3):231-7.
(16) Dhillon S. Tramadol/paracetamol fixed-dose combination: a review of its use in the management of moderate to severe pain. Clinical drug investigation. 2010 Oct;30(10):711-38.
(17) Nna VU, Osim EE. Testicular toxicity following separate and combined administration of PDE5 inhibitors and opioid: Assessment of recovery following their withdrawal. Andrologia. 2017 Aug;49(6):e12669
(18) Nakhaee S, Farrokhfall K, Miri-Moghaddam E, *et al*. The effects of naloxone, diazepam, and quercetin on seizure and sedation in acute on chronic tramadol administration: an experimental study. Behavioral and Brain Functions. 2021 May 29;17(1):5.
(19) Radbruch L, Glaeske G, Grond S, *et al*. Topical review on the abuse and misuse potential of tramadol and tilidine in Germany. Substance abuse. 2013 Jul;34(3):313-20.
(20) Raffa RB, Stone DJ. Unexceptional seizure potential of tramadol or its enantiomers or metabolites in mice. Journal of pharmacology and experimental therapeutics. 2008 May 1;325(2):500-6.
(21) Beyaz SG, Sonbahar T, Bayar F, Erdem AF. Seizures associated with low-dose tramadol for chronic pain treatment. Anesthesia, essays and researches. 2016 May;10(2):376-8.
(22) Hassamal S, Miotto K, Dale W, Danovitch I. Tramadol: understanding the risk of serotonin syndrome and seizures. The American journal of medicine. 2018 Nov 1;131(11):1382-e1.
(23) Afshari R, Afshar R, Mégarbane B. Tramadol overdose: review of the literature. Médecine Intensive Réanimation. 2011 Aug 25;20(5):436-41.
(24) Abadinsky H. Drug use and abuse: A comprehensive introduction. 7th ed. Wadsworth, Cengage Learning, Belmont, USA; 2011:475pp.
(25) United Nations Office on Drugs and Crime. World Drug Report 2020. *United Nations* publication, accessed 20 February2020, https://wdr.unodc.org/wdr2020/en/index2020.html
(26) Sabry NA, Rabie MA, Sedrak AE, Khafagy WE. The National Research on Addiction. 1st ed. General Secretariat of Mental Health and Addiction Treatment, MoHP, Cairo, Egypt; 2015:80pp.
(27) Shamloul RM, Elfayomy NM, Ali EI, *et al*. Tramadol-associated seizures in Egypt: Epidemiological, clinical, and radiological study. Neurotoxicology. 2020 Jul;79:122-6.
(28) USP U. Pharmacopoeia-national formulary [USP 38 NF 33]. Rockville, Md United States Pharmacopeial Convention. 2015.
(29) British Pharmacopoeia Commission. British Pharmacopeia 2016: volume III. London:TSO; 2016.
(30) Martyn-St James M, Cooper K, Kaltenthaler E, *et al*. Tramadol for premature ejaculation: a systematic review and meta-analysis. BMC urology. 2015 Dec; 15:6.
(31) Xin ZC, Zhu YC, Yuan YM, *et al.* Current therapeutic strategies for premature ejaculation and future perspectives. Asian Journal of Andrology. 2011 Jul;13(4):550-57.



ISSN: 3009-7061