ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article DOI: 10.5281/zenodo.15729141

Determination of tramadol in pharmaceutical preparations by GC-MS method

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ABSTRACT

In this study, a gas chromatography-mass spectrometry (GC-MS) method was developed to analyze tramadol in both pure form and pharmaceutical formulations. Diclofenac served as the internal standard. The linearity of the GC-MS method was validated within a concentration range of 0.05-5.0 µg mL^{-1}. The intra- and inter-day relative standard deviations were found to be below 1.18% and 3.51%, respectively. The detection and quantification limits for the GC-MS method were determined to be 0.015 and 0.045 µg mL^{-1}, respectively. Stability testing revealed that tramadol remained stable in solutions after 24 hours of incubation at room temperature or after 72 hours of storage at 4 °C and -20°C. Under the selected assay conditions, tablet excipients did not cause any interference. Furthermore, the method proved to be effective in quantifying tramadol and ensuring the consistency of formulation content in commercial tramadol dosage forms.

Keywords: Tramadol, GC-MS, Validation, Tablet.

INTRODUCTION

For more than 40 years, tramadol has been prescribed often throughout the world; nonetheless, there is a chance that it will be abused and trafficked. Because of this, drug analysis is a crucial component of contemporary analytical chemistry and has several legal and socially significant ramifications. Tramadol is an analogue of codeine that is 2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol or 4-phenylpiperidine. The effectiveness of tramadol, a commonly prescribed analgesic, in reducing moderate to severe pain has drawn a lot of attention in the field of pain treatment.[1]

Large oral dosages of tramadol may have a euphoric affect similar to that of oxycodone. Prevalent reasons of tramadol poisoning have been shown to be attempted suicide (52-80 %), abuse (18-31 %) and inadvertent intoxication (1–11 %). A history of chronic tramadol usage or opioid dependency was linked to at least 20% of cases of tramadol poisoning.[2]

Tramadol is metabolized extensively in the liver as a prodrug. Forensic investigation, therapeutic monitoring, medication interactions, and pharmacokinetic evaluation of tramadol all depend on an understanding of its metabolic metabolism.[3] It is usually administered orally, and within two hours, it is distributed after being fully absorbed. The therapeutic range for TD blood concentrations is 0.1-0.3 mg L^{-1}. Furthermore, it has been said that blood readings of 1 and 2 mg L^{-1}, respectively, have the potential to be lethal and harmful.[4,5]

Due to the early stage of metabolism and the fact that 6% of the population has slow-acting CPY2D6, which has a little diminished analgesic effect, the bioavailability ranges from 65% to 70%. Compounds N and O-desmethylated from tramadol are produced.[6–9]

Tramadol and its metabolites were determined using a variety of analytical techniques, such as mass spectrometry detection[10–12] or gas chromatography with nitrogen selective. Recently, novel separation techniques have been introduced, such as capillary electrophoresis[13] and performance liquid chromatography (HPLC) approaches with electrochemical[14], mass spectrometry[15–18] or fluorescence detection.[19–24]

As of right now, there is no published technique for identifying tramadol in pharmaceutical formulations using GC-MS. Therefore, the current study's goal was to create a GC-MS method that is specific, sensitive, accurate, and precise for analyzing tramadol in pharmaceutical formulations. The International Conference on Harmonization (ICH) criteria were followed in the complete validation of the proposed technique with regard to detection and quantification limits, precision, accuracy, linearity, specificity, stability, and recovery parameters.[25]

Received 04.03.2025
Revised 07.04.2025
Accepted 10.06.2025
Publication Date 24.06.2025

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Cite this article: Yılmaz B., Alkan E. Determination of tramadol in pharmaceutical preparations by GC-MS method. *NanoEra*. 2025;5(1):22-27

METHODS

Chemicals

Tramadol hydrochloride and diclofenac were purchased from Sigma-Aldrich (St. Louis, MO, USA). Contramal Retard tablet drug (100 mg tramadol hydrochloride) was obtained from the pharmacy (Erzurum, Turkey). Every chemical was of the analytical variety.

GC-MS System and chromatographic conditions

Agilent Technologies, Palo Alto, California, provided the Agilent 6890N gas chromatography system, which was outfitted with an Agilent chemstation, a 7673 series autosampler, and a 5973 series mass selective detector for the chromatographic study. The separation was performed using an HP-5 MS column (30 m × 0.25 mm I.D., USA) with a film thickness of 0.25 µm. Splitless injection was utilized with helium as the carrier gas at a flow rate of one milliliter per minute, and the injector had a capacity of one µL. The solvent delay was set to two minutes, the electron energy for the MS detector was set to 70 eV, and the transfer line temperature was set to 280°C.

The MS detector's characteristics included an electron energy of 70 eV, a solvent delay of 3 minutes, and a transfer line temperature of 290°C. The MS was employed in scan mode (m/z 40-500) for qualitative analysis and in selected ion monitoring (SIM) mode (m/z 214 for internal standard (IS) diclofenac and m/z 58 for tramadol) for quantitative analysis.

Preparation of the standard and quality control solutions

A 100 µg mL^{-1} methanol concentration was used to generate the tramadol stock solution, which was then chilled to -20°C. After that, the mixture was gradually diluted with methanol to provide standard working solutions with tramadol concentrations of 0.05, 0.10, 0.25, 0.5, 1, 2, 3, 4 and 5 µg mL^{-1}. A stock solution containing 50 µg mL-1 of IS was produced in methanol. Before being used, all of the solutions were allowed to come to room temperature from their 4°C storage. The standard working solution of tramadol was added to aliquots to create the quality control (QC) solutions at final concentrations of 0.75, 2.5 and 4.5 µg mL^{-1}, containing 0.1 mL IS (1 µg mL^{-1}).

Procedure for pharmaceutical preparations

Using the mass of the Contramal Retard tablets, the average tablet mass was computed. Following that, they underwent homogenization, fine grinding, and meticulous weighing of a portion of the powder. The necessary amount of methanol was then poured to them in a 100 mL brown measuring flask in order to dilute the powder. After sonicating the mixture for a minimum of fifteen minutes to facilitate dissolution, it was filtered using a Whatman No. 42 paper. After a suitable amount of filtrate was further diluted with methanol to ensure that the final solution's tramadol concentration was within the working range.

Data analysis

A computer program, SPSS 15.0, (SPSS Inc., Chicago, IL, USA) was utilized to perform the statistical analyses. The tramadol standard curve and subsequent calculations were derived using regression analysis techniques. The mean and standard deviation of the results were provided.

RESULTS

Method development and optimization

Based on its chemical properties, the tramadol assay method was developed. The capillary column used in this experiment, coated with 5% phenyl and 95% dimethylpolysiloxane, is an excellent choice for separation because the analytes elute as symmetrical peaks throughout a broad concentration range. For the GC oven, various temperature ranges were examined.

The ideal temperature program for a successful separation was identified at the study's conclusion. The temperature programs for the GC oven were as follows: The increases in temperature were as follows: first, from 70°C to 250 °C at 35°C min^{-1}, which was held for one minute; next, to 290°C at 20°C min^{-1}, which was kept for one minute.

It was decided to use the splitless injection mode. Early accuracy and linearity studies of the method also demonstrated the reproducibility of the 1 µL injection volume and the significance of the peak response at the selected analytical concentration.

Validation of the method

The goal of method validation is to prove that the approach is appropriate for the purpose for which it was designed, as specified in ICH guidelines. The technique was verified for linearity, accuracy, precision stability, recovery and system applicability.[25]

Specificity

To assess the specificity of the method, potential interferences between tramadol and the excipients were investigated. For the quantitative analysis using GC-MS, electron impact ionization with selected ion monitoring (SIM) was applied, targeting the ion at m/z 58 for tramadol. The mass spectrum of tramadol is shown in Figure 1. The retention time of tramadol in the GC-MS analysis was approximately 7.4 minute, with a clear and distinct peak, as illustrated in Figure 2.

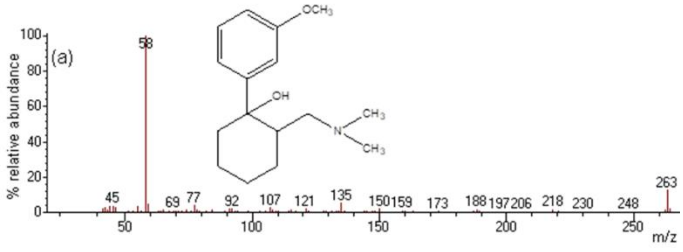

Figure 1. MS spectrum of tramadol (1.0 mg mL^{-1})

Linearity

An analytical procedure is considered linear when the test results are directly related to the analyte concentration in the sample within a specific range, either in a straightforward manner or through a well-defined mathematical transformation. Initially, linearity should be assessed visually by analyzing a plot of the signal against the analyte concentration. If the relationship appears linear, the test results should be validated

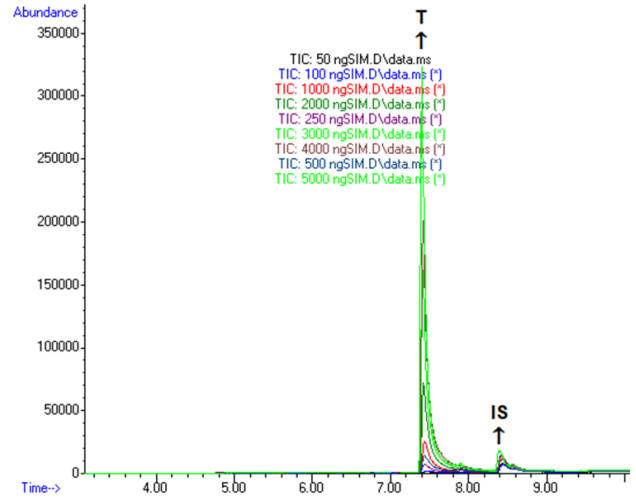

Figure 2. GC-MS chromatogram of tramadol (0.05, 0.10, 0.25, 0.5, 1, 2, 3, 4 and 5 mg mL^{-1}) and IS (1.0 mg mL^{-1})

using appropriate statistical methods, such as calculating a regression line using the least squares method. In some cases, a mathematical adjustment of the test results may be required to ensure linearity between the analyte's response and its concentration.

For the purpose of estimating the degree of linearity mathematically, information from the regression line itself may be useful. It is necessary to present the regression line's slope, residual sum of squares, y-intercept, and correlation coefficient. Analysis was done on standard solutions containing 1.0 µg mL^{-1} of IS and 0.05 - 5.0 µg mL^{-1} of tramadol. The standard curve (Fig. 3) was created by plotting the concentration of ramadol on the X-axis and the peak area ratio of tramadol and IS on the Y-axis.

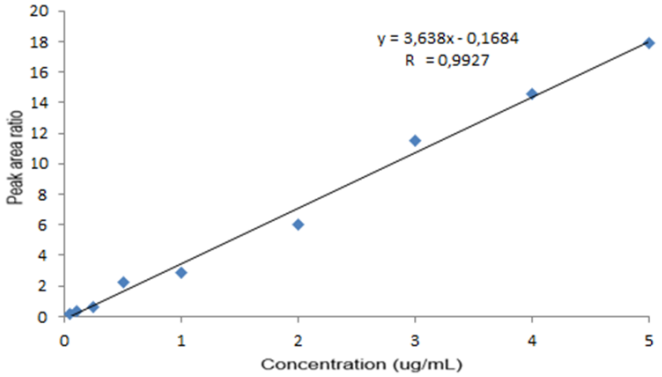

Figure 3. The standard curve of tramadol (0.10-5.0 µg mL^{-1})

Using the least squares regression approach to construct the linear regression analysis, the linearity was assessed. The regression equation was computed from the calibration graphs (Table 1).

Table 1. Linearity of tramadol

Parameter	GC-MS
Linearity (µg mL^{-1})	0.05-5.0
Regression equation	y=3.638x-0.1684
Correlation coefficient	0.9927
LOD (µg mL^{-1})	0.015
LOQ (µg mL^{-1})	0.045

[a]Based on six calibration curves, y=peak area ratio, x=concentration of tramadol

Accuracy and precision

Accuracy is described as how closely test results produced by the procedure resemble the actual value. By using known, added amounts of analyte in the experiment, it is frequently stated as the percent recovery. The exactness of the analytical process is gauged by accuracy. The true values were utilized to determine the variances of the obtained results, which were then reported as a % accuracy.

Precision is the ability to reproduce an analytical process across multiple homogeneous sample samplings. The standard deviation or relative standard deviation of a set of measurements is typically used to represent the precision of an analytical procedure. A measure of precision could be the analytical method's repeatability or degree of reproducibility under typical operating conditions.

By analyzing the QC samples six times, the assay method's accuracy for intra- and inter-day fluctuations was assessed. The precision and accuracy results for the QC samples both within and between day runs are displayed in Table 2. Between 1.18% and 3.51% ranged the precision, and between 0.67% and 3.20% the accuracy.

Table 2. Precision and accuracy of tramadol

	Intra-day			Inter-day		
Added (µg mL^{-1})	Found ± SD[a] (µg/mL)	Precision % RSD[b]	Accuracy[c]	Found ± SD[a] (µg/mL)	Precision % RSD[b]	Accuracy[c]
0.75	0.77 ± 0.027	3.51	2.67	0.74 ± 0.014	1.89	-1.33
2.5	2.42 ± 0.070	2.89	-3.20	2.52 ± 0.037	1.47	0.80
4.5	4.53 ± 0.053	1.18	0.67	4.51 ± 0.067	1.49	2.22

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation, Accuracy[c]: % relative error: (found-added)/addedx100

Limits of detection (LOD) and quantification (LOQ)

Tramadol's LOD and LOQ values were ascertained by evaluating various tramadol solutions and calculating the signal-to-noise ratio for every analyte. The concentration providing a signal-to-noise ratio of roughly 3:1 is the LOD, while the concentration providing a signal-to-noise ratio of roughly 10:1 with an RSD of less than 10% with triplicate analysis is the LOQ. The GC-MS technique yielded LOD and LOQ values of 0.015 and 0.045 µg mL^{-1}, respectively.

Stability

Standard solutions representing the lowest, middle, and highest points of the calibration curves were prepared independently to evaluate the stability of tramadol under varying temperatures and time conditions. The stability tests revealed that the samples remained stable for 8 hours at room temperature, for short-term storage at 4°C and -20°C, and for 72 hours at these lower temperatures for long-term storage. The stability study results, shown in Table 3, indicated no significant degradation, with values falling within the acceptable range of 90 to 110 percent. The findings are detailed in Table 3.

Table 3. Stability of tramadol in solution (n=6)

	Intra-day			Inter-day	
Conc. (µg mL^{-1})	Room temperature 24 h	Refrigeratory 4°C, 24 h	Frozen -20°C, 24 h	Refrigeratory 4°C, 48 h	Frozen -20°C, 48 h
0.5	100.7 ± 2.76	99.4 ± 3.78	99.2 ± 2.41	99.4 ± 3.47	98.5 ± 3.43
2.5	101.7 ± 2.17	100.6 ± 3.57	97.3 ± 3.75	98.2 ± 2.17	101.2 ± 2.78
5.0	99.3 ± 3.473	101.3 ± 2.74	98.4 ± 3.46	100.2 ± 2.71	99.4 ± 2.74

Table 4. Recovery of tramadol in pharmaceutical preparation

		Intra-day		Inter-day	
Pharmaceutical preparation	Added (µg mL^{-1})	Found ± SD[a] (µg mL^{-1})	% Recovery % RSD[b]	Found ± SD[a] (µg mL^{-1})	% Recovery % RSD[b]
Contramal Retard (1.5 µg mL^{-1})	0.5	0.49 ± 0.019	98.7 (4.07)	0.49 ± 0.016	97.9 (3.27)
	1.5	1.49 ± 0.071	99.2 (4.78)	1.46 ± 0.040	98.7 (2.77)
	3.5	3.51 ± 0.145	100.3 (4.13)	3.47 ± 0.178	99.2 (5.12)

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation

Recovery

Recovery values were obtained by adding different amounts of pure drug to tablet samples, which had already been pre-analyzed, within the analytical concentration range of the proposed method. The additional doses of each drug were then determined using the described procedure. The results from the recovery tests were considered satisfactory and are presented in Table 4.

System suitability

Before each validation run, a system suitability test was performed on the chromatographic system. Efficiency and area relative standard deviation were calculated for each of the five suitable injections. The five suitability injections' average was used to quantify the check standard. The efficiency was ≥3562 and the %RSD was ≤1.47% for all sample analysis.

Procedure for pharmaceutical preparations

The 100 mg tramadol-containing Contramal Retard tablet was precisely weighed and finely powdered. A measured amount of the powder was dissolved in 50 milliliters of methanol. Then, the solution was transferred to a 100 mL volumetric flask and filled to the mark with methanol. After properly diluting the tablet solutions, they were filtered using a Whatman filter to achieve a final concentration within the linear range of the GC-MS method (Figure 4). The tramadol concentration was determined using the calibration curve.

DISCUSSION

In biological samples, GC-MS is a potent technology that can assess low amounts of analytes quantitatively and with great specificity. High-resolution capillary GC has not been utilized as much as HPLC lately. Tramadol and O-desmethyltramadol could, however, be detected simultaneously because to its naturally high resolving power, high sensitivity, and outstanding precision and accuracy. GC in conjunction with MS also reduced the detection limits to ng levels. Through an investigation of the peak interference from the exogenous chemicals, the specificity of the approach was confirmed. Figure 2 displayed a representative tramadol and IS chromatogram.

El-Sayed et al.[10] developed a GC-MS method for the determination of tramadol and O-desmethyltramadol in human urine following α-glucuronidase hydrolysis and liquid-liquid extraction, utilizing positive electron impact ionization. The total run time for the proposed method was 12.6 minutes. The calibration curve was found to be linear within the 10-1000 ng mL^{-1} range. Intra-assay precision for tramadol and O-desmethyltramadol ranged from 1.29% to 6.48%, while inter-assay precision ranged from 1.28% to 6.84%. The intra-assay accuracy for both tramadol and O-desmethyltramadol was between 91.79% and 106.89%.

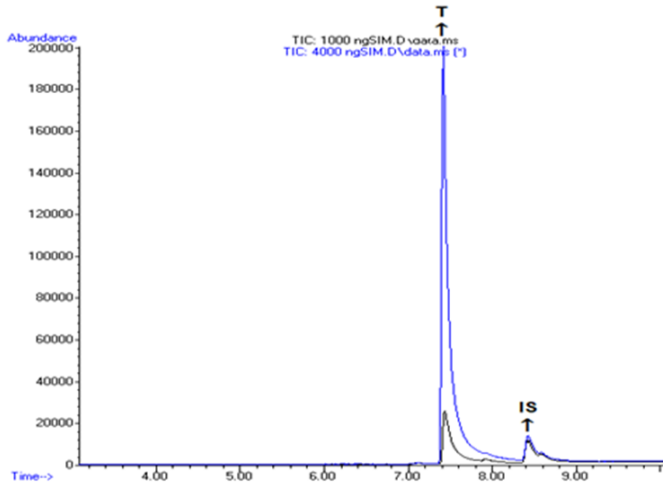

Figure 4. The chromatogram of Contramal Retard tablet solution (1.0 and 4.0 µg mL^{-1})

Using solvent bar microextraction, Ghasemi[11], a GC-MS technique has been developed for the determination of tramadol in various biological samples. With a 4.5 % RSD, the detection limit was 0.02 µg L^{-1}. A solid phase extraction technique was used to remove tramadol from plasma. In addition to being the most thorough approach, this one can extract tramadol in a single extraction process. The entire run time of the procedure is 15 minutes.

Biological samples have low sensitivity and selectivity when it comes to UV detection, even though tramadol and its metabolite molecules include a benzene ring.[26] Currently, mass spectrometry and fluorescence are the only two types of detectors that have attained low quantification levels. The lowest tramadol quantification level[12,19,21] using any of them was around 1.0 ng mL^{-1}, indicating a lower detection limit of 0.2 - 0.5 ng mL^{-1}.[12,19]

Using tandem mass detection using LC techniques, the amount of tramadol and O-desmethyltramadol in human plasma is revealed.[15–18] According to Ceccato et al.[15] tramadol and O-desmethyltramadol in human plasma were evaluated using the LC technique with tandem mass detection. The tramadol calibration curve utilizing the LC-MS/MS method was linear between 20 and 10,000 ng mL^{-1}. The RSD value indicated the intra- and inter-day precision of less than 6.4%, and relative error, or accuracy, was better than 6.1%. More sensitive would be LC-MS/MS detection, although at the moment, not all laboratories can afford it.

In the literature, tramadol was recovered from plasma using a solid phase extraction technique.[26] In addition to being the most thorough approach, this one can extract tramadol in a single extraction step. In comparison to the research published by Ardakani et al.[27] and Moore et al.[28], the mean recovery is higher. The proposed method, with a total run time of 8.0 minutes, is faster than the previously reported GC-MS methods for tramadol and its metabolites.[29,30] Additionally, it achieves adequate sensitivity for both O-desmethyltramadol and tramadol without the need for derivatization, offering an improvement over the methods previously published.[28]

This method's sensitivity was found to be sufficient for pharmacokinetic studies when applied to tablet samples. When compared to the previously mentioned methods, the current approach presents several advantages. It is either superior or at least on par with the methods described in earlier studies.[23,27–30]

CONCLUSION

In this study, a GC-MS method was developed and validated for the determination of tramadol, offering a quick and straightforward approach. The chromatographic method satisfies all essential criteria, including accuracy, linearity, recovery, and precision, making it a reliable and practical technique. Due to the 9-minute run time, a large number of samples can be analyzed in a short period. Therefore, this method is suitable not only for routine formulation and raw material analysis but also for analyzing samples during accelerated stability studies.

Peer-review: Externally peer-reviewed.
Author Contributions: Concept - B.Y., E.A.; Supervision - B.Y.; Resources - B.Y., E.A.; Materials – B.Y., E.A.; Data Collection and/or Processing - E.A., B.Y.; Analysis and/or Interpretation - B.Y., E.A.; Writing Manuscript - B.Y., E.A.; Critical Review – B.Y.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support

REFERENCES

1. Gibson TP. Pharmacokinetics, efficacy, and safety of analgesia with a focus on tramadol HCl. *Am J Med*. 1996;101:S47-S53. doi:10.1016/S0002-9343(96)00138-6
2. Shipton EA. Tramadol—Present and Future. *Anaesth Intensive Care*. 2000;28(4):363-374. doi:10.1177/0310057X0002800403
3. Cooper RJ. Over-the-counter medicine abuse – a review of the literature. *J Subst Use*. 2013;18(2):82-107. doi:10.3109/14659891.2011.615002
4. Almér Herrnsdorf E, Holmstedt A, Håkansson A. Tramadol misuse in treatment-seeking adolescents and young adults with problematic substance use – Prediction of treatment retention. *Addict Behav Reports*. 2022;16:100446. doi:10.1016/j.abrep.2022.100446
5. Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in Tramadol: Pharmacology, Metabolism, and Misuse. *Anesth Analg*. 2017;124(1):44-51. doi:10.1213/ANE.0000000000001683
6. Allegaert K, Anderson BJ, Verbesselt R, et al. Tramadol disposition in the very young: an attempt to assess in vivo cytochrome P -450 2D6 activity. *Br J Anaesth*. 2005;95(2):231-239. doi:10.1093/bja/aei170
7. Goeringer KE, Logan BK, Christian GD. Identification of Tramadol and its Metabolites in Blood from Drug-Related Deaths and Drug-Impaired Drivers. *J Anal Toxicol*. 1997;21(7):529-537. doi:10.1093/jat/21.7.529
8. Quetglas EG, Azanza JR, Cardenas E, Sádaba B, Campanero MA. Stereoselective pharmacokinetic analysis of tramadol and its main phase I metabolites in healthy subjects after intravenous and oral administration of racemic tramadol. *Biopharm Drug Dispos*. 2007;28(1):19-33. doi:10.1002/bdd.526
9. Ardakani YH, Rouini M. Pharmacokinetics of tramadol and its three main metabolites in healthy male and female volunteers. *Biopharm Drug Dispos*. 2007;28(9):527-534. doi:10.1002/bdd.584
10. El-Sayed A-AY, Mohamed KM, Nasser AY, Button J, Holt DW. Simultaneous determination of tramadol, O-desmethyltramadol and N-desmethyltramadol in human urine by gas chromatography–mass spectrometry. *J Chromatogr B*. 2013;926:9-15. doi:10.1016/j.jchromb.2013.02.019
11. Ghasemi E. Optimization of solvent bar microextraction combined with gas chromatography mass spectrometry for preconcentration and determination of tramadol in

biological samples. *J Chromatogr A*. 2012;1251:48-53. doi:10.1016/j.chroma.2012.06.060

12. Sha YF, Shen S, Duan GL. Rapid determination of tramadol in human plasma by headspace solid-phase microextraction and capillary gas chromatography–mass spectrometry. *J Pharm Biomed Anal*. 2005;37(1):143-147. doi:10.1016/j.jpba.2004.09.050
13. Rudaz S, Veuthey JL, Desiderio C, Fanali S. Simultaneous stereoselective analysis by capillary electrophoresis of tramadol enantiomers and their main phase I metabolites in urine. *J Chromatogr A*. 1999;846(1-2):227-237. doi:10.1016/S0021-9673(99)00028-X
14. Valle M, Pavón J., Calvo R, Campanero M., Trocóniz I. Simultaneous determination of tramadol and its major active metabolite O-demethyltramadol by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl*. 1999;724(1):83-89. doi:10.1016/S0378-4347(98)00547-7
15. Ceccato A, Vanderbist F, Pabst J-Y, Streel B. Enantiomeric determination of tramadol and its main metabolite O-desmethyltramadol in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 2000;748(1):65-76. doi:10.1016/S0378-4347(00)00318-2
16. Musshoff F, Madea B, Stuber F, Stamer UM. Enantiomeric Determination of Tramadol and O-Desmethyltramadol by Liquid Chromatography-Mass Spectrometry and Application to Postoperative Patients Receiving Tramadol. *J Anal Toxicol*. 2006;30(7):463-467. doi:10.1093/jat/30.7.463
17. Musshoff F, Trafkowski J, Kuepper U, Madea B. An automated and fully validated LC-MS/MS procedure for the simultaneous determination of 11 opioids used in palliative care, with 5 of their metabolites. *J Mass Spectrom*. 2006;41(5):633-640. doi:10.1002/jms.1021
18. Zhu T, Ding L, Guo X, Yang L, Wen A. Simultaneous Determination of Tramadol and Acetaminophen in Human Plasma by LC–ESI–MS. *Chromatographia*. 2007;66(3-4):171-178. doi:10.1365/s10337-007-0268-9
19. Campanero MA, Calahorra B, García-Quetglás E, Escolar M, Honorato J. High-performance liquid chromatographic assay for simultaneous determination of tramadol and its active metabolite in human plasma. Application to pharmacokinetic studies. *Chromatographia*. 1998;48(7-8):555-560. doi:10.1007/BF02466649
20. Nobilis M, Kopecký J, Květina J, et al. High-performance liquid chromatographic determination of tramadol and its O-desmethylated metabolite in blood plasma. *J Chromatogr A*. 2002;949(1-2):11-22. doi:10.1016/S0021-9673(01)01567-9
21. Campanero MA, García-Quetglas E, Sádaba B, Azanza JR. Simultaneous stereoselective analysis of tramadol and its primary phase I metabolites in plasma by liquid chromatography. *J Chromatogr A*. 2004;1031(1-2):219-228. doi:10.1016/j.chroma.2003.11.034
22. Pedersen RS, Brøsen K, Nielsen F. Enantioselective HPLC method for quantitative determination of tramadol andO-desmethyltramadol in plasma and urine: Application to clinical studies. *Chromatographia*. 2003;57(5-6):279-285. doi:10.1007/BF02492397
23. Rouini M-R, Ardakani YH, Soltani F, Aboul-Enein HY, Foroumadi A. Development and validation of a rapid HPLC method for simultaneous determination of tramadol, and its two main metabolites in human plasma. *J Chromatogr B*. 2006;830(2):207-211. doi:10.1016/j.jchromb.2005.10.039
24. LI Q, Wang R. Simultaneous analysis of tramadol, metoprolol and their metabolites in human plasma and urine by high performance liquid chromatography. *Chin Med J (Engl)*. 2006;119(23). https://journals.lww.com/cmj/fulltext/2006/12010/simultaneous_analysis_of_tramadol,_metoprolol_and.12.aspx
25. Validation of analytical procedures: Text and Methodology. In: *International Conference on Harmonization (ICH). Commission of the European Communities*. Vol 2. ; 1996.
26. Gan S., Ismail R. Validation of a high-performance liquid chromatography method for tramadol and o-desmethyltramadol in human plasma using solid-phase extraction. *J Chromatogr B Biomed Sci Appl*. 2001;759(2):325-335. doi:10.1016/S0378-4347(01)00237-7
27. Ardakani YH, Rouini M-R. Improved liquid chromatographic method for the simultaneous determination of tramadol and its three main metabolites in human plasma, urine and saliva. *J Pharm Biomed Anal*. 2007;44(5):1168-1173. doi:10.1016/j.jpba.2007.04.012
28. Moore C, Rana S, Coulter C. Determination of meperidine, tramadol and oxycodone in human oral fluid using solid phase extraction and gas chromatography–mass spectrometry. *J Chromatogr B*. 2007;850(1-2):370-375. doi:10.1016/j.jchromb.2006.12.008
29. Cheng P-S, Lee C-H, Liu C, Chien C-S. Simultaneous Determination of Ketamine, Tramadol, Methadone, and their Metabolites in Urine by Gas Chromatography-Mass Spectrometry. *J Anal Toxicol*. 2008;32(3):253-259. doi:10.1093/jat/32.3.253
30. Chytil L, Štícha M, Matoušková O, Perlík F, Slanař O. Enatiomeric determination of tramadol and O-desmethyltramadol in human urine by gas chromatography–mass spectrometry. *J Chromatogr B*. 2009;877(20-21):1937-1942. doi:10.1016/j.jchromb.2009.04.042