ORIGINAL ARTICLE

Chronotherapeutic Press-Coated Tablets of Tramadol Hydrochloride: *In Vivo* Evaluation

Ramya Krishna Talakoti[1,2], Suresh Bandari[3], A. Jaswanth[4]

[1]Department of Pharmacy, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India, [2]Department of Pharmaceutics, Talla Padmavathi Pharmacy College, Warangal, Telangana, India, [3]Department of Pharmaceutics and Drug Delivery School of Pharmacy, University of Mississippi, Oxford, Mississippi, United States, [4]Department of Pharmacology, Mother Terasa College of Pharmacy, Mettusalai, Tamil Nadu, India

Abstract

Objective: The purpose of this study is to conduct *in vivo* pharmacokinetic study of Tramadol Hydrochloride (TH) press-coated tablets (PCTs) in rabbit plasma using reversed-phase high-performance liquid chromatography (RP-HPLC) technique. **Materials and Methods:** A Phenomenex bond clone reverse phase C18 (4.6 × 150 mm, 5 µm) column and mobile phase acetonitrile – 0.01 M phosphate buffer (30:70% v/v) containing 0.1 % triethylamine with 1 ml/min flow rate were used for development and validation of RP-HPLC method, further used to calculate various pharmacokinetic parameters of TH core tablets and PCT in rabbits which received TH core and PCTs. **Results and Discussion:** The developed method showed a linearity in range of 10–800 ng/ml ($r^2 = 0.9997$), good precision and accuracy with acceptable relative standard deviation values, repeatability, and reproducibility. The drug TH extracted with recovery in range of 96.20–98.29%. The pharmacokinetic study of PCT showed 5 h lag time with drug release. The C_{max} and T_{max} of core tablet and PCTs were 409.86 ± 40.46 ng/ml at 0.75 h and 289.46 ± 32.58 ng/ml at 10 h, respectively. Area under curve$_{(0-t)}$ of core and PCTs was 1531.26 ± 499.09 ngh/ml and 2293.48 ± 521.8 ngh/ml, respectively. **Conclusion:** Based on results, PCTs provided better chronotherapeutic release characteristics with drug release after a specified lag time using polymer blend of HPMC E50 and HPMC E100 as release modifiers for therapy of Rheumatoid arthritis.

Key words: Press-coated tablets, reversed-phase high-performance liquid chromatography, tramadol hydrochloride

INTRODUCTION

Rheumatoid arthritis (RA) is a severe systemic auto-immune disease which produces inflammation in the joints, surrounding tissue and other body organs.[1] RA is characterized by diurnal variations in circulating cytokines levels and interleukin-6 or tumor necrosis factor[2] and has symptoms such as pain in joints, warmth, and stiffness of the body which are produced early morning[3] on waking or after extended inactivity.[4] Increased stiffness in early morning is the common sign of the condition and it usually lasts beyond an hour necessitating the use chronomodulated drug release for maximum therapeutic benefit. Hence, press-coated tablet (PCT) has been one of the important approaches for releasing the drug after defined lag time.[5]

Tramadol HCL (TH) is an opioid agonist and synthetic, centrally acting analgesic. It is used to relieve moderate to severe pain that has low selectivity for µ receptor. It inhibits norepinephrine and serotonin reuptake, further alters the transmission of pain signals.[6] TH showed a therapeutic index in range of 100–300 ng/ml. It has a 5–6 h half-life. The recommended daily dose of TH is 50–100 mg taken orally in divided doses. It has a high oral bioavailability and is well absorbed throughout the gastrointestinal tract. Certain water-soluble drug formulations have rapid release rate and are more prone to produce harmful drug concentrations when taken orally.[7] TH is a BCS Class-I medication, stating it has a high solubility and permeability and is likely to produce harmful concentrations with nausea

Address for correspondence:
Ramya Krishna Talakoti, Department of Pharmaceutics, Talla Padmavathi Pharmacy College, Warangal, Telangana, India. E-mail: ramyatalakoti@gmail.com

Received: 14-01-2022
Revised: 18-03-2022
Accepted: 26-03-2022

and vomiting as side effects, which can be eliminated using pulsatile delivery to delay the drug's release.[8] TH has been assessed in pharmaceutical,[9] urine,[10] and blood plasma[11,12] using high-performance liquid chromatography (HPLC) with ultraviolet (UV),[9-11,13-15] fluorescence,[12,16] or electrochemical[17] detection.

In the literature survey carried out, there are only some estimation procedures of TH in rabbit plasma by reversed-phase HPLC (RP-HPLC) method.[9-15] Hence, an attempt was made to develop a RP-HPLC method which is more accurate, precise. The purpose of this research was to establish and validate a sensitive and reliable RP-HPLC method for pharmacokinetic evaluation of TH in rabbit plasma and to carry out *in vivo* studies of PCTs.

MATERIALS AND METHODS

Materials and reagents

TH was a gift sample from Hetero Drugs, Hyderabad, India. Triethylamine (TEA) and methanol (HPLC grade) and acetonitrile were procured from Merck. All other chemicals were of analytical quality. Distilled water was used to prepare the buffer solution. Chronotherapeutic tablets are prepared by press coating technique. The composition, formulation, and *in vitro* evaluation of optimized PCT (PCT4) were described in our previous work.[18]

Instruments

The HPLC system consisted of HPLC (Shimadzu) equipped with pump (LC-20AD), UV–Visible detector (SPD-20A) and Rheodyne injector with 20 µl sample loop. The parameters of HPLC were controlled by laboratory solution software. The detector had a distinct band fixed to 271 nm, that was the wavelength employed for quantification, and it was configured to scan from 200 to 500 nm. Electronic balance (Shimadzu-AY220), bath sonicator (Biotechnics), and digital pH meter (Systronics) were used.

Chromatographic requirements

A Phenomenex bond clone RP C18 column with particle size of 5 µm (4.6 × 150 mm) was used as the analytical column. The temperature of column was at 25°C. A vestel computer with laboratory solution software was used to operate the HPLC system and collect data. The mobile phase flow rate and injection volume were 1 ml/min and 20 µl, respectively, and was detected at 271 nm.[19,20]

The mobile phase composition was changed using several combinations of acetonitrile – 0.01 M phosphate buffer (PB) (10:90, 20:80, 30:70, and 40:60 %v/v). The peak tailing is avoided using sufficient concentration of 0.01 M PB. The composition of mobile phase comprising acetonitrile – 0.01 M PB (30:70 %v/v) with 0.1% TEA was optimized. The pH of mobile phase was adjusted to 3 by addition of orthophosphoric acid and further filtered through 0.47 µm membrane filter and degassed 20 min by ultrasonication. The mobile phase base line was chosen giving the best area count with least band tailing for drug. Wash out period was done with mobile phase (methanol – water, 70:30% v/v). Asymmetry and retention on column were decreased by adding TEA. TH retention time was 5.44 min for our RP-HPLC system [Figure 1].

Preparation of standard solutions and plasma standards

A standard stock solution (1 µg/ml) of TH in mobile phase was prepared and stored at 4°C. A series of seven standard solutions (10, 50, 100, 200, 400, 600, and 800 ng/ml) were made and the calibration graph was plotted in 10–800 ng/ml range for TH (n = 6).

Drug-free rabbit plasma was spiked with various working standard solutions to make plasma standard solutions which were further diluted to give concentration range of 10–800 ng/ml. From different stock solution, plasma control samples were prepared in concentration of 10, 100, 200, 400, and 600 ng/ml.

Plasma sample preparation procedure

One-step liquid-liquid extraction was used to prepare plasma samples. In a 4 ml test tube, rabbit plasma (0.25 ml) was mixed with 1M NaOH (0.01 ml) and then extracted with 1.25 ml ethyl acetate, followed by vertexing (10 min) and centrifugation (10000 rpm, 10 min). The supernatant organic phase was taken into conical flask, further extracted again with another 1.25 ml of ethyl acetate. The organic phase was dried under a moderate air and reconstituted in 250 ml of a mobile phase.[19,20]

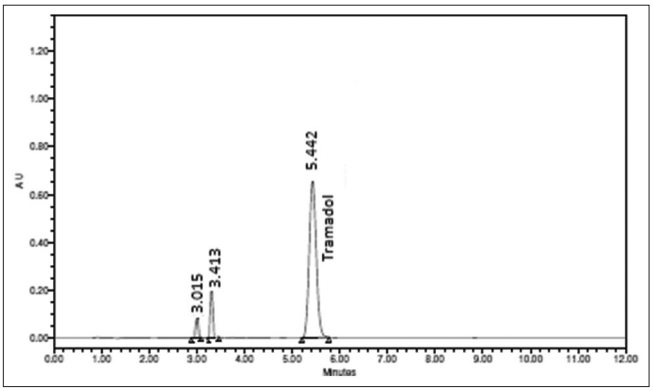

Figure 1: Chromatogram of tramadol hydrochloride standard solution (100 ng/ml) in reversed-phase high-performance liquid chromatography system

Validation

Developed analytical technique is validated for detection of TH in rabbit plasma for following parameters: System suitability, linearity, accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), recovery, and stability.[21]

System suitability

Standard solution of TH (100 ng/ml) was injected 6 times in HPLC system and suitability parameters were calculated and compared with those mentioned by the Center for Drug Evaluation and Research (CDER).[22]

Linearity

The least square technique was used to measure seven level calibration series comprising six analyzes at each concentration level.[20]

Specificity

Specificity is the capability to measure the analyte precisely and accurately without interferences from blank or plasma. Diluent and plasma were injected into HPLC system to compare the interference using direct comparison technique.

Precision and accuracy

Spiked plasma samples were assayed at four different concentrations (10, 200, 400, and 600 ng/ml) as intra-day and inter-day for tramadol in mobile phase and the relative standard deviation (RSD = 100 × S.D/mean) was computed to determine the precision.[23] Accuracy was determined from the method by the percent relative error which was calculated as [(Concentration found-known concentration) × 100/known concentration].[23]

LOQ and detection

The minimum plasma concentration in the calibration graph that could be determined with acceptable precision on a regular basis was designated as the LOQ (RSD <20%).[24] LOD was defined as a signal-to-noise ratio of 3.

Recovery

The recovery of TH in rabbit plasma was estimated at four levels of the calibration graph by comparing data acquired by direct injection of standard solutions to data obtained after the whole extraction process. The peak areas obtained from freshly prepared sample extracts were compared to those obtained by direct injection of standard solutions of the same concentrations.[23]

Stability

Stock solution (10 ng/ml) was stored at 4°C and 25°C for at least 1 month and studied for degradation (on day, 7, 14, 21, and 28). Stability of stock solution is indicated by no significant degradation occurred at 1 month.[20]

Preclinical study

Animals

Preclinical study was conducted using eight male adult healthy New Zealand rabbits (mean weight ± SD, 3.1 ± 0.4 kg). These animals were starved for 12 h before administration of tablets and have free access to fresh water during this period. The animals were given access to the food 12 h after receiving the tablets. The study was carried out in compliance with all ethical criteria for laboratory animal investigations, which were authorized by the Institution's Ethics Committee (1505/PO/a/08 CPCSEA 2020) and were carried out in accordance with the regulations and guidelines for the care and use of laboratory animals.[25]

Study design

Preclinical study was performed on eight New Zealand male adult rabbits by dividing into two groups. All animals received each core tablet and PCT orally. Core tablet and PCTs were administered orally by a gastric intubation tube.[26] 20 ml of water were given with tablet to ensure tablet which was swallowed and entered the stomach at the time of administration. Rabbits are kept in retainer, while blood samples (1.5 ml) were collected from catheter remaining in the marginal ear vein, before administration and 0.25, 0.5, 0.75, 1, 2, 6, 10, 12, and and 24 h following administration of core tablets and 30 min, 1, 2, 3, 4, 5, 6, 8,10, 12, and 24 h following administration of PCTs. Blood samples were placed in collection tubes having lithium heparin and centrifuged at 2880× g for 10 min, after which the plasma was frozen at −30°C until analysis.[27]

Pharmacokinetic study

A graph of the mean plasma concentration versus time profile has been plotted. The maximum plasma concentration (C_{max}), time required to reach peak plasma levels (T_{max}), and other pharmacokinetic parameters, that is, area under curve from zero to time (AUC_{0-t}), AUC from zero to infinity ($AUC_{0-\infty}$), elimination rate constant (k_e), and elimination half-life ($t_{1/2kel}$) were computed using Kinetica software (Kinetica 20000 version 3, Inna phase corporation, 2000).

RESULTS AND DISCUSSION

Validation

System suitability

System suitability parameters were calculated using the optimized parameters compared with those mentioned by the CDER.[22] Each validation run's system suitability method acceptance parameters were within the acceptable criteria [Table 1].

Linearity

The high correlation coefficients (r^2) achieved by the regression line revealed the linearity of the calibration graph. The mean regression equation is y = 6529.3x -20355 [Figure 2]. The linearity of TH was in the range of 10–800 ng/ml (r^2 = 0.9997). The regression equations were calculated from the calibration graph.

Specificity

When plasma and diluent were injected into an HPLC system, there was no interference from the diluent and plasma peaks at the standard TH peak retention time [Figure 3a and b].

Table 1: System suitability parameters of tramadol hydrochloride (100 ng/ml)

System suitability parameter	Observed value	Acceptance criteria
Percentage RSD of peak area	1.38	<2
Capacity factor	1.50	>2
Theoretical plate count	638,780	>2000
Tailing factor/ asymmetry	0.9	≤2

RSD: Relative standard deviation

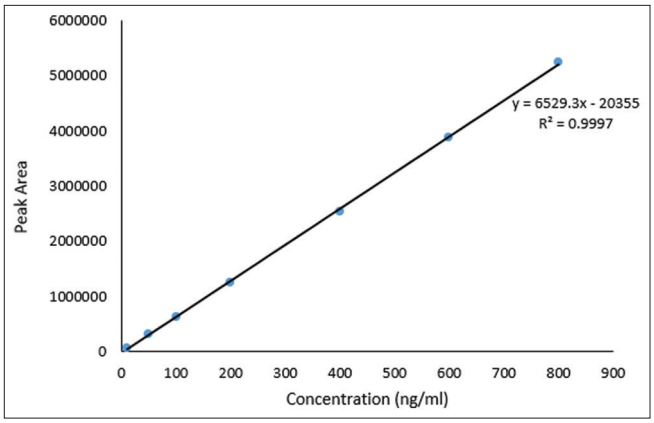

Figure 2: Linearity of tramadol hydrochloride

Precision and accuracy

Precision and accuracy were calculated according to formulae and expressed as RSD. Precision and accuracy investigations revealed acceptable RSD values, relative errors ≤10, and good accuracy ≤10 for both intra- and inter-day studies (n = 6) [Table 2].

LOQ and detection

LOD of TH in rabbit plasma was 5 ng/ml (signal-to-noise ratio = 2.98) [Figure 4], while the LOD of TH in rabbit plasma was 5 ng/ml (signal-to-noise ratio = 2.98).

Recovery

The TH extraction recovery of tramadol in rabbit plasma was assessed using rapid one-step liquid-liquid extraction procedure. Due to its strong polarity and volatility, ethyl acetate was chosen as the solvent for our liquid–liquid extraction approach.[23] The solvent ethyl acetate provided excellent recovery, with absolute tramadol recoveries of 96–98% from rabbit plasma [Table 3]. No interfering or ghost peaks were detected in the blank plasma and plasma samples.

Stability

The stability of TH at various temperatures revealed no significant degradation occurred and it is stable at 4 and 25°C for at least 1 month [Table 4].

Pharmacokinetic study

The TH plasma concentration at various time intervals following oral administration of both core tablet and PCT is shown in graph of mean plasma concentration versus time profile [Figure 5]. It reveals that PCT maintained therapeutic plasma concentration even up to 10 h as compared to core tablets, which showed fast decrease in drug concentration with time as tablets disintegrated very fast in GIT and resulted in quick absorption. The therapeutic index of TH is in range of 100–300 ng/ml. The peak plasma concentration (C_{max}) and time to reach peak concentration (T_{max}) of core tablet and PCT

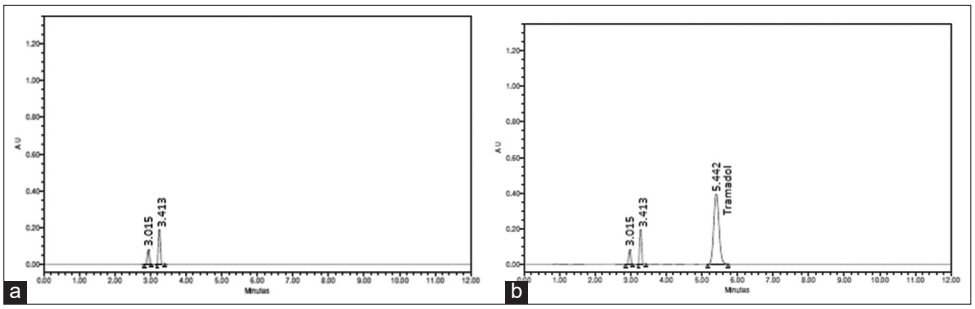

Figure 3: Chromatogram of (a) drug free rabbit plasma (blank) and (b) standard tramadol hydrochloride (10 ng/ml) in rabbit plasma

Table 2: Precision and accuracy data for determination of tramadol hydrochloride in rabbit plasma (n=6)

Intra-day				Inter-day		
Added concentration (ng/ml)	Found concentration (ng/ml)	RSD (precision, %)	Accuracy (relative error, %)	Found concentration (ng/ml)	RSD (precision, %)	Accuracy (relative error, %)
10	9.62	0.99	−3.8	9.81	1.07	−1.9
200	194.15	3.71	−2.92	193.46	3.00	−3.27
400	395.47	2.06	−1.13	396.29	2.57	−0.92
600	593.72	3.27	−1.04	591.92	1.99	−1.46

RSD: Relative standard deviation

Table 3: Assay recovery data of tramadol hydrochloride in rabbit plasma

Added concentration (ng/ml)	Found concentration (ng/ml)	Recovery ratio (%)	Accuracy relative error (%)	RSD (%)
10	9.62	96.20	−3.8	0.78
200	192.26	96.13	−3.87	3.42
400	390.64	97.66	−2.34	1.50
600	589.79	98.29	−1.70	1.38

RSD: Relative standard deviation

Table 4: Stability of tramadol hydrochloride stock solution (10 ng/ml) under different storage conditions

Temperature (°C)	Day				
	0	7	14	21	28
4	101	99	96	94	93
25	102	100	98	95	92

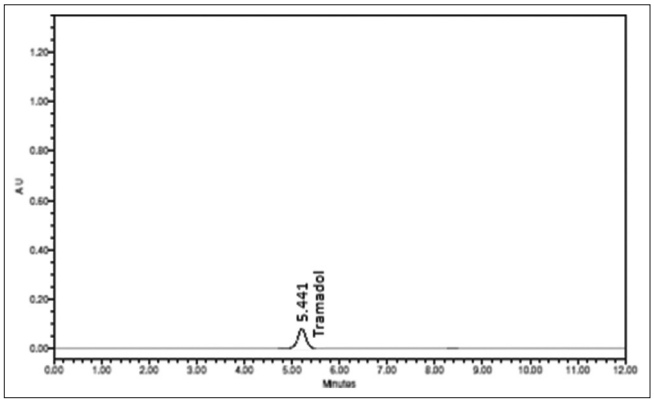

Figure 4: Chromatogram for limit of detection

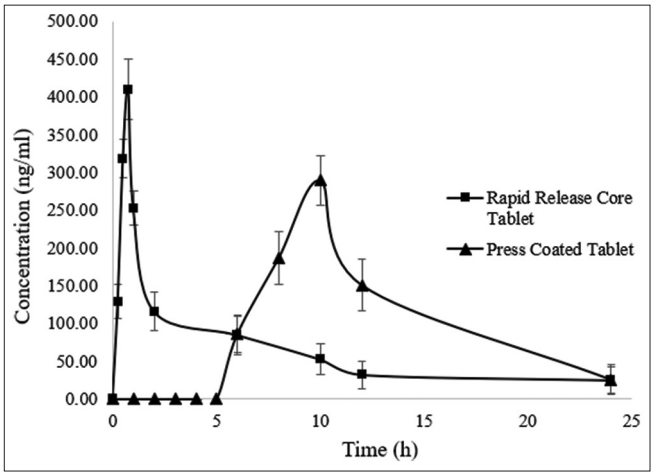

Figure 5: Plasma concentration-time profiles of tramadol hydrochloride after oral administration of core tablet and selected press-coated tablet

were 409.86 ± 40.46 ng/ml, 289.46 ± 32.58 ng/ml, and 0.75 ± 0.02 h, 10 ± 0.01 h, respectively. Lower C_{max} of PCT (with in therapeutic index) reveals minimized danger of exceeding the maximum therapeutic concentration. Higher T_{max} of PCT reveals lag time of 5 h followed by drug release which is suitable for chronotherapy of RA. AUC_{0-24} of core and PCTs was 1531.26 ± 499.0 ngh/ml and 2293.48 ± 521.8 ngh/ml, respectively. Higher AUC_{0-24} of PCT reveals superiority of PCT over core tablet in providing slow drug release with a lag time of 5 h and improved bioavailability. $AUC_{0-\infty}$ of core and PCTs was 1803.94 ± 433.86 ngh/ml and 2657.193 ± 519.8 ngh/ml. Elimination rate constant (k_e) and half-life ($t_{1/2kel}$) of core tablet and PCT were found to be 0.0908 h^{-1}, 7.63 h and 0.0711 h^{-1}, and 9.74h^{-1}, respectively.

CONCLUSION

The developed RP-HPLC method was simple, specific, accurate, and precise. This method was suitable for estimation of TH content in rabbit plasma from PCTs. Pharmacokinetic studies revealed lower C_{max} and delayed T_{max} of PCT compared to core tablet indicating minimized danger of exceeding therapeutic concentration and lag time of 5 h followed by complete drug release in 10 h. Based on

the results, this PCT is suitable as chronomodulated delivery system for therapy of RA. Hence, PCTs with desired lag time followed by drug release were developed which provided better chronotherapeutic release characteristics using HPMC E50 and HPMC E100 as release modifiers.

ACKNOWLEDGMENTS

Ramya Krishna T (author) acknowledge Hetero drugs, Hyderabad, India for providing gift samples. The author is grateful to Dr. Suresh B and Dr. Jaswanth A (guides) for their valuable support and for guiding me to carry out this research work. The author is more thankful to Dr. J. Venkateshwar Rao (principal) and management of Talla Padmavathi Pharmacy College for providing the facilities. The author is more thankful to Dr. Satyanaraya Patnaik for giving me valuable information.

REFERENCES

1. Rothschild BM, Rothschild C, Helbling M. Unified theory of the origins of erosive arthritis: Conditioning as a protective/directing mechanism? J Rheumatol 2003;30:2095-102.
2. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, *et al.* Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004;350:2572-81.
3. Arvidson NG, Gudbjörnsson B, Elfman L, Rydén AC, Tötterman TH, Hällgren R. Circadian rhythm of serum interleukin-6 in rheumatoid arthritis. Ann Rheum Dis 1994;53:521-4.
4. Bierma-Zeinstra SM, Oster JD, Bernsen RM, Verhaar JA, Ginai AZ, Bohnen AM. Joint space narrowing and relationship with symptoms and signs in adults consulting for hip pain in primary care. J Rheumatol 2002;29:1713-8.
5. Survase S, Kumar N. Pulsatile drug delivery: Current scenario. Curr Res Inf Pharm Sci 2007;8:27-33.
6. Rubin BR, Talent JM, Kongtawelert P, Pertusi RM, Forman MD, Gracy RW. Oral polymeric N-acetyl-D-glucosamine and osteoarthritis. J Am Osteopath Assoc 2001;101:339-44.
7. Lata MS, Panchal A, Shelat PK, Zaveri MN. Development of sustained release matrix tablet of tramadol hydrochloride using natural gums. Int J Pharm Pharm Sci 2012;4:153-9.
8. Parag AK, Mahendra J, Santosh BJ, Satish VS, Pramod VK. Development and evaluation of press coated tablets for chronopharmaceutical drug delivery using gellable and permeable polymers. Pharm Lett 2010;2:482-97.
9. Zaghloul IY, Radwan MA. High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms. J Liq Chromatogr Relat Technol 1997;20:779-87.
10. Elsing B, Blaschke G. Achiral and chiral high-performance liquid chromatographic determination of tramadol and its major metabolites in urine after oral administration of racemic tramadol. J Chromatogr 1993;612:223-30.
11. Ceccato A, Chiap P, Hubert P, Crommen J. Automated determination of tramadol enantiomers in human plasma using solid-phase extraction in combination with chiral liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;698:161-70.
12. Nobilis M, Pastera J, Anzenbacher P, Svoboda D, Kopecký J, Perlík F. High-performance liquid chromatographic determination of tramadol in human plasma. J Chromatogr B Biomed Appl 1996;681:177-83.
13. Yeh GC, Sheu MT, Yen CL, Wang YW, Liu CH, Ho HO. High-performance liquid chromatographic method for determination of tramadol in human plasma. J Chromatogr B Biomed Sci Appl 1999;723:247-53.
14. Gan SH, 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;759:325-35.
15. Gan SH, Ismail R, Wan Adnan WA, Wan Z. Method development and validation of a high-performance liquid chromatographic method for tramadol in human plasma using liquid-liquid extraction. J Chromatogr B Analyt Technol Biomed Life Sci 2002;772:123-9.
16. Overbeck P, Blaschke G. Direct determination of tramadol glucuronides in human urine by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl 1999;732:185-92.
17. Valle M, Pavón JM, Calvo R, Campanero MA, Trocóniz IF. 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;724:83-9.
18. Ramya KT, Suresh B, Jaswanth A. Chronotherapeutic press-coated tablets of tramadol hydrochloride: Formulation and *in vitro* evaluation. Asian J Pharm 2017;11:S358-66.
19. Rouini MR, 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 Analyt Technol Biomed Life Sci 2006;830:207-11.
20. Küçük A, Kadioğlu Y, Celebi F. Investigation of the pharmacokinetics and determination of tramadol in rabbit plasma by a high-performance liquid chromatography-diode array detector method using liquid-liquid extraction. J Chromatogr B Analyt Technol Biomed Life Sci 2005;816:203-8.
21. FDA Guideline for Submitting Samples and Analytical Data for Method Validations, Rockville, MD, USA;

February, 1987. Available from: http://www.fda.gov/cder.guidance. Last accessed on 2021 Dec 03].
22. Reviewer Guidance: Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), Food and Drug Administration; November, 1994.
23. Kucuk A, Kadioglu Y. Method development and validation of a HPLC-DAD method for determination of tramadol in human plasma in two different solvent media. J Pharm Sci 2005;30:196-203.
24. Buick AR, Doig MV, Jeal SC, Land GS, McDowall RD. Method validation in the bioanalytical laboratory. J Pharm Biomed Anal 1990;8:629-37.
25. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Official Journal of the European Union L276/33, 20.10.2010; 2010.
26. Ranjan OP, Nayak UY, Reddy MS, Dengale SJ, Musmade PB, Udupa N. Osmotically controlled pulsatile release capsule of montelukast sodium for chronotherapy: Statistical optimization, *in vitro* and *in vivo* evaluation. Drug Deliv 2014;21:509-18.
27. Szkutnik-Fiedler D, Balcerkiewicz M, Sawicki W, Grabowski T, Grześkowiak E, Mazgalski J, *et al. In vitro – In vivo* evaluation of a new oral dosage form of tramadol hydrochloride-controlled-release capsules filled with coated pellets. Acta Pol Pharm 2014;71:469-75.

Source of Support: Nil. **Conflicts of Interest:** None declared.