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ICH guideline practice: application of validated RP-HPLC-DAD method for determination of tapentadol hydrochloride in dosage form

Deepti Jain¹ and Pawan K Basniwal^{1,2*}

Abstract

Background: Tapentadol is a novel centrally acting analgesic. There is no method for the determination of drug content in dosage form without any interference of any excipients and without using the diethylamine content in the mobile phase with UV detection.

Methods: A simple, precise, and accurate new reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated as per International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines to determine tapentadol hydrochloride in tablet dosage form. It was successfully eluted at 5.34 min by mixture of 0.1% formic acid in water and acetonitrile (75:25) on C18 column (250 mm × 4.6 mm, 5 μm) at the flow of 1 ml/min.

Results: Drug content was determined in between 99.79% and 100.33% with standard deviation of 0.217 without using dimethylamine with UV detection.

Conclusions: The validated RP-HPLC method may be successfully applied for assay, dissolution studies, bio-equivalence studies, as well as routine analysis in pharmaceutical industries.

Keywords: RP-HPLC; Tapentadol hydrochloride; Tablet

Background

Tapentadol (TAP), chemically 3-[(1*R*,2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol (Figure 1), is a novel centrally acting analgesic with a dual mode of action: μ -opioid receptor agonism and noradrenaline reuptake inhibition. This combination of dual action effect not only provides effective analgesia in a broad range of acute and chronic pain conditions but also provides an 'opioid-sparing' effect, i.e., to lower the dose of TAP required to produce a given level of analgesia. TAP also exerted analgesic effects in patients undergoing moderate-to-acute, inflammatory, and chronic neuropathic pain (Schneider et al. 2010; Vadivelu et al. 2011; Wade and Spruill 2009). However, in veterinary practice, its use is still uncertain and controversial (Giorgi 2012a). Recently, a comprehensive review on tapentadol counting the

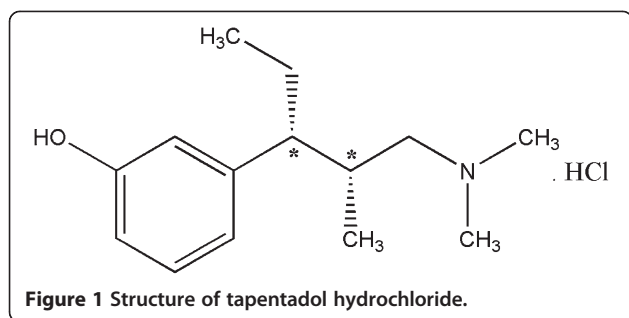
recent trends in synthesis, related substances, analytical methods, pharmacodynamics, and pharmacokinetics has been published (Jain and Basniwal 2013).

Only few studies were conducted to determine TAP, *viz.* in urine (Coulter et al. 2010), in urine and oral fluid (Bourland et al. 2010) by liquid chromatography-mass spectrometry (LC-MS), and in canine plasma by high-performance liquid chromatography (HPLC) with spectrofluorimetric detection (Giorgi et al. 2012b) and pharmacokinetic studies in dogs (Giorgi et al. 2012c). UV detection was performed in most pharmaceutical industries, while spectrofluorimetric detector is not widely applicable. Longer saturation time is needed when amino derivative (diethylamine or triethylamine) modifier is used as a component of mobile, which is not recommended for routine analysis of drug content in the pharmaceutical industry. It also causes bonus troubles during analysis such as erratic baselines and poor peak shape (Synder et al. 1997). In this context, very complex mobile phases were used for the separation of

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TAP such as phosphate buffer and triethylamine (Marathe et al. 2013), potassium dihydrogen orthophosphate and potassium hydroxide (Kathirvel et al. 2013), and phosphate buffer with glacial acetic acid and triethylamine (Sherikar and Mehta 2012). As we have experienced, the working range for HPLC methods is near 10 µg/ml, while the mentioned phases have very high working ranges at 75 to 300 µg/ml (Kathirvel et al. 2013) and 5 to 100 µg/ml (Sherikar and Mehta 2012).

Thus, there is still a gap in research work; there is no method for the determination of TAP in dosage form, which claims that there is no interference of any excipients in determination without using complex mobile phase and with higher sensitivity. The new reverse-phase HPLC (RP-HPLC) method was developed and validated as per International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines without using diethylamine content in the mobile phase with DAD detection, which is simple, rapid, precise, and accurate for the determination of TAP in tablet dosage form.

Methods

Instrumentation and chromatograph

The HPLC chromatograph used was Agilent Infinity 1260 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a 1260 binary pump VL (400 bar), 1260 manual injector (600 bar), Rheodyne 7725i seven-port sample injection valve with a 20-µl fixed loop, ZORBAX Eclipse Plus C18 (250 mm × 4.6 mm, 5 µm), 1260 DAD VL, 20-Hz detector, a standard flow cell (10 mm, 13 µl, and 120 bar; OpenLab CDS EZChrom Ed. Workstation), and 50-µl syringe (FN, LC tip). All weighing for analysis was performed on a Shimadzu electronic analytical balance AX-200 (Kyoto, Japan). Water used for analysis was prepared by triple distillation assembly. All dilutions, mobile phase and other solutions, used for the analysis were filtered through a 0.2-µm nylon filter (Ultipor®N66 Nylon 6,6 membrane, Pall Sciences, Pall India Pvt. Ltd. Mumbai, India).

Chemicals and reagent

The working standard used was TAP which was supplied by Ranbaxy Laboratories Limited, Gurgaon, Harayana, as a gift sample. Tablets of TAP (TRANSDOL 50, Lupin Ltd. Mumbai, India) were procured from the local market. Triple distilled water was prepared from distillation assembly. Formic acid and acetonitrile were procured from Merck Specialties Private Limited Mumbai, India. The mobile phase was prepared from the combination of formic acid (0.1%) and acetonitrile.

Sample preparation

Accurately weighed 50 mg of TAP was dissolved in triple distilled water to prepare stock I (1,000 µg/ml) in 50-ml volumetric flask. Stock II (100 µg/ml) was prepared from stock I, which was used to prepare further dilutions containing 0.2, 0.4, 0.8, 1.2, 1.6, and 2 µg/ml. All dilutions were filtered through 0.2-µm nylon filter (Ultipor®N66 Nylon 6,6 membranes, Pall Sciences).

Chromatography

The filtered dilutions were chromatographed by the set of conditions on Agilent Infinity 1260 series. The mixture of 0.1% formic acid and acetonitrile (75:25) was used as mobile phase for the elution of the drug on ZORBAX Eclipse Plus C18 column (250 mm × 4.6 mm, 5 µm) at 1 ml/min of flow rate. TAP was successfully eluted at 5.8 min with a run time of 7 min, and detection was performed by photodiode array detector (PDA) at 272 nm.

Method validation

According to the ICH guidelines (ICH Q2A 1994; ICH Q2B 1996), the developed method was validated to assure the reliability of the results of analysis for different parameters *viz.* linearity, range, accuracy, precision, robustness, ruggedness, limit of quantization (LOQ), limit of detection (LOD), and specificity. Linearity was determined by serial dilutions (0.2 to 2 µg/ml) of the TAP in distilled water in triplicates. The range of TAP was validated between 0.8 and 1.6 µg/ml with triplicates of dilutions. Accuracy was determined by recovery method by spiking the standard solution to the pre-analyzed samples. This procedure was repeated six times. Precision of the method was studied under the head of repeatability and intermediate precision. The six replicates of 0.8 µg/ml were chromatographed subsequently to assure the repeatability. Intermediate precision was determined by day-to-day analysis variation and analyst-to-analyst variation in the linearity range. Robustness of the method was studied with variation in temperature (20°C, 25°C, and 30°C) and content of formic acid (0.1%) in aqueous phase variation by a 5% change. Serial dilutions of TAP from 1 to 1,000 ng/ml were chromatographed, which was repeated three times to determine LOD and LOQ. Specificity was ascertained by

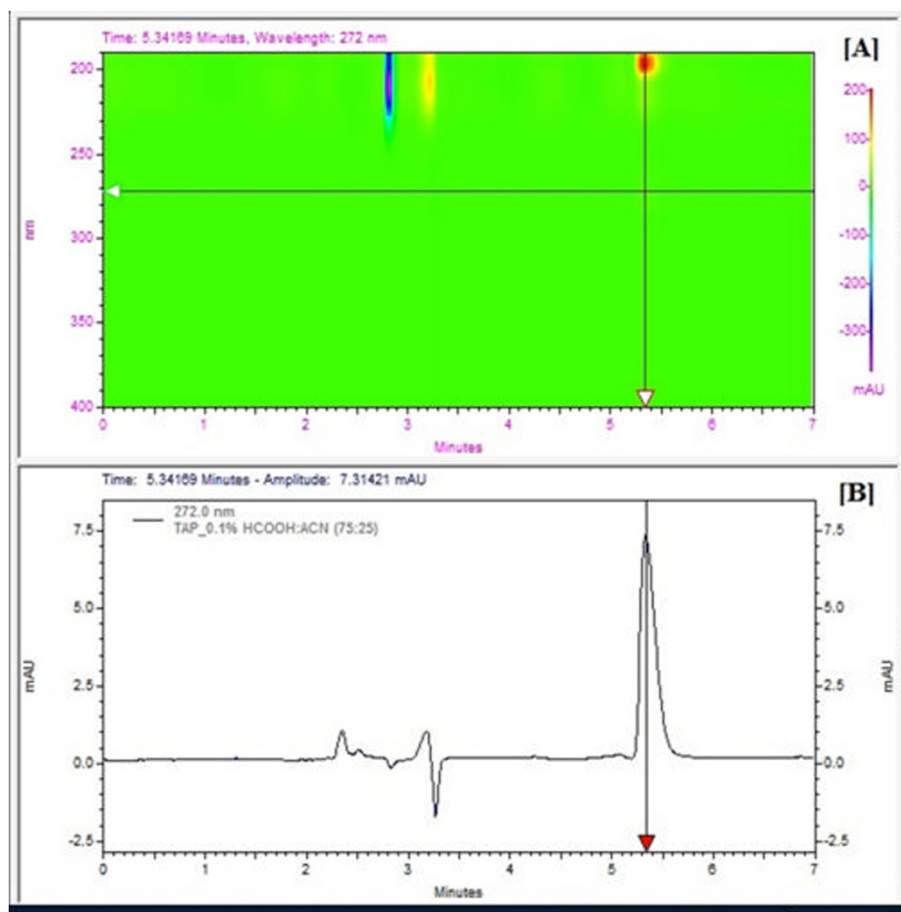


Figure 2 Counter plot (A) and chromatogram (B) of TAP. TAP is in 0.1% formic acid and acetonitrile (75:25) at 5.34 min.

degrading the drug sample in alkaline medium. Sample solution stability was demonstrated by analyzing six replicates of 0.8 $\mu\text{g}/\text{ml}$ TAP standard samples at different time intervals (0, 12, 24, 36, and 48 h) with freshly prepared mobile phase on each time.

Analysis of dosage form

Powdered tablets were weighed equivalent to 50 mg of TAP and sonicated to dissolve drug content in triple distilled water. The sonicated solution was filtered through Whatman filter (no. 41), and different serial dilutions were

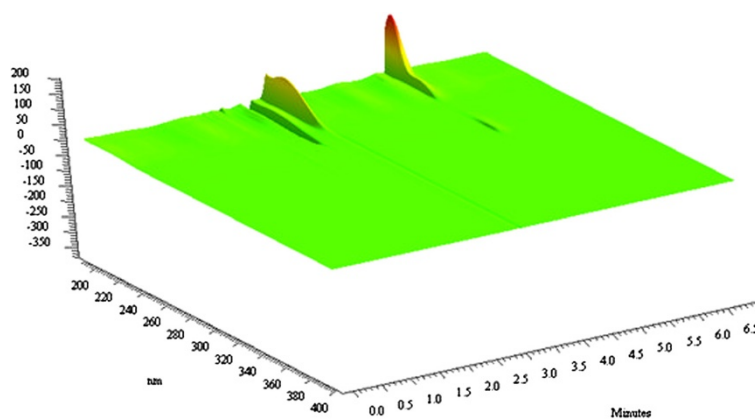


Figure 3 Three-dimensional view of chromatogram of TAP in 0.1% formic acid and acetonitrile (75:25) at 5.34 min.

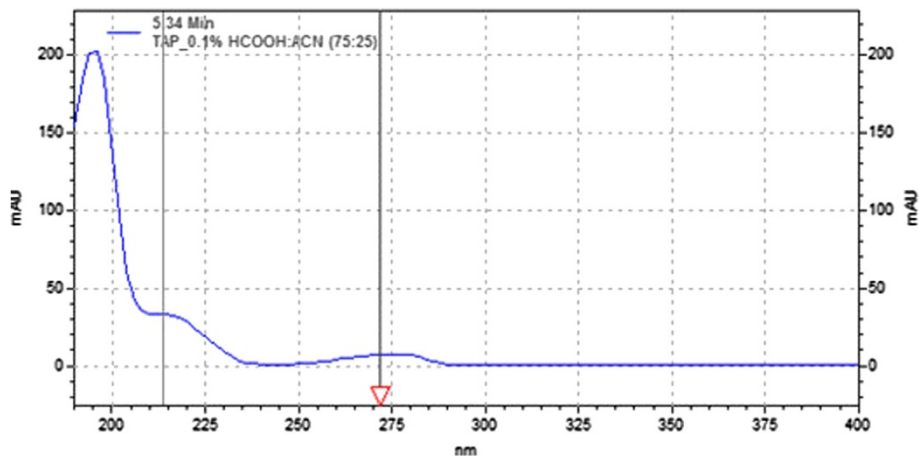


Figure 4 Spectrum of TAP at 5.34 min in 0.1% formic acid and acetonitrile (75:25).

prepared by subsequent dilution with triple distilled water. The dilutions were chromatographed, and percentages of drug content in tablets were determined by extrapolating the AUC values on regression line.

Results and discussion

Optimization of chromatography

The RP-HPLC method was optimized with the objective of developing a simple, precise, accurate, and fast assay method for TAP. On the basis of chemistry and solubility of TAP, the initial mixture of water and acetonitrile (50:50) was made to elute TAP on C18 column (250 mm × 4.6 mm, 5 μm) at the flow of 1 ml/min. The splitting of peak was observed, which indicates that the drug ionizes in the present mobile phase and the peak was highly broadened. Now, the pKa values (9.34 and 10.45) of the TAP have been taken into consideration to design the mobile phase (Synder et al. 1997), so the acidic pH should be favored to elute the drug in a single peak with an acceptable peak shape because in acidic pH, it should be present in

nonionic form. Formic acid (0.1%) in water and acetonitrile (50:50) was run to elute TAP. The drug was eluted with a diluent peak which was splitting in nature. When aqueous content was decreased, more splitting was observed with diluent peaks, i.e., acidic content in the mobile phase should be increased. Thus, aqueous phase (acidic content) was increased to 70%; the single peak without splitting was observed which was near the diluent peak with acceptable peak shape but with tailing. Finally, TAP was better resolved from the diluent peak without splitting with acceptable peak shape (no tailing and fronting) in 0.1% formic acid in water and acetonitrile (75:25). Thus, we have excluded the use of amino derivative (diethylamine or triethylamine) as modifier of the mobile phase.

It was clearly shown in Figure 2A,B that the diluent peak comes in between 2.5 and 3.5 min and is well resolved from the TAP peak (5.34 min). Three-dimensional view (Figure 3) of the chromatogram also confirmed that there was no peak around the TAP elution time. The above mobile phase composition enabled the peak shape and

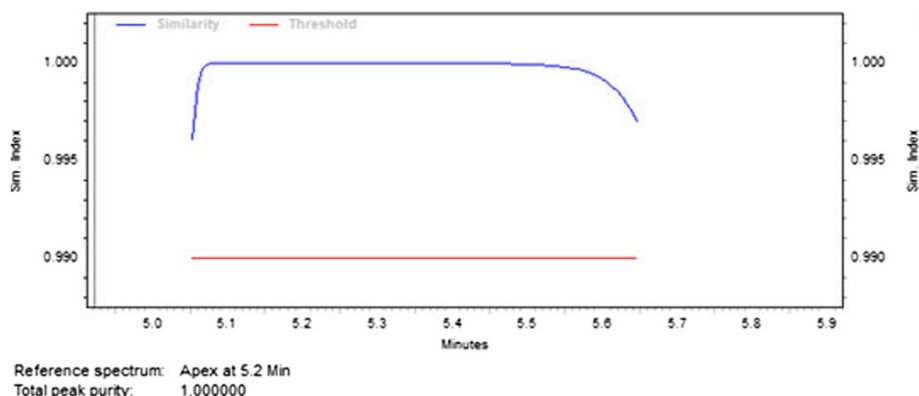


Figure 5 Peak purity curve of TAP at 5.34 min in 0.1% formic acid and acetonitrile (75:25).

elution of the drug, and in this separation, the UV detector has good spectra of the drug. The detection wavelength was confirmed at 272 nm by the spectrum of TAP at an elution time of 5.34 min (Figure 4), while previously reported methods have different detection, *viz.* spectrofluorimetric and LC-MS methods, which were costly than UV detection. The peak purity curve at the elution time of the TAP (Figure 5) indicated that there was no interference with the peak of TAP as the peak purity of the TAP was one unit.

System suitability parameters

System suitability parameters were analyzed to check the system performance consistency. For system suitability parameters, six replicates of high quality control sample of TAP was injected, and column performances like tailing factor, retention time, and number of theoretical plates were observed (Table 1); values of percentage of relative standard deviation (%RSD) for these parameters were found within the acceptance criteria of system performance. Higher theoretical plates (21,930) with a lower %RSD value indicate good separation of TAP on the C18 column in described chromatographic conditions. Tailing factors for most peaks should fall between 0.9 and 1.4, with a value of 1.0 indicating a perfectly symmetrical peak. Tailing factor for TAP was in between the range; thus, the TAP's peak was in symmetrical shape. The capacity factor was 16.44 ± 0.222 , i.e., TAP has sufficient opportunity to interact with the stationary phase resulting in differential migrations. The co-elution of impurities or degradants is generally investigated by examining the peak purity using a PDA detector. Here, the peak purity at 5.34 min was 1.0; there was no interference with elution of TAP at retention time, or nothing was co-eluting along with TAP at 5.34 min. Thus, all system suitability parameters were within the acceptance criteria.

Linearity and range

Linearity was evaluated by analyzing different concentrations of the standard solutions of the TAP. Response was a linear function of concentration over the range of 0.2 to 2 µg/ml which was used as the working range of the method. Peak area and concentration were subjected to

Table 1 System suitability parameters for TAP

Parameters	Value for TAP ^a	SD	%RSD
Number of theoretical plates	21,930	61.64	0.281
Tailing factor	1.12	0.051	0.956
Capacity factor	16.44	0.222	1.352
RT (min)	5.34	0.037	0.698
Peak purity	1.00	0.000	0.000

RT retention time, SD standard deviation, %RSD percentage of relative standard deviation. ^aMean of six values.

Table 2 Validation parameters of RP-HPLC method for TAP

Parameters	Values ^a ± SD, ± %RSD
Linearity	0.2 to 2 µg/ml
Regression equation	AUC = 18,374.93x - 62.59
Correlation coefficient	$r^2 = 0.9998 \pm 0.001, \pm 0.100$
Response ratio	2,377.778 ± 1.510, ± 0.063
Range	0.8 to 1.6 µg/ml ± 1.23, ± 0.02
Accuracy	100.05 ± 0.841, ± 0.841
Precision	
Repeatability	100.07 ± 0.319, ± 0.319
Intermediate precision	
Inter-day	99.89 ± 0.205, ± 0.205
Analyst to analyst	99.74 ± 0.125, ± 0.125
Robustness	
Temperature (20°C, 25°C, 30°C)	100.21 ± 0.75, ± 0.748
Formic acid concentration (±5%)	100.12 ± 0.54, ± 0.539
LOQ	8 ng/ml ± 0.93, ± 0.063
LOD	1 ng/ml ± 0.78, ± 0.067
Specificity	Ascertained by analyzing standard drug and samples of equivalent concentration
Stability in sample solution	
Response ratio	184.16 ± 1.83, ± 0.057

LOD limit of detection, LOQ limit of quantization. ^aMean of six replicates.

linear least-squares regression analysis to calculate the calibration equation and correlation coefficient (Table 2). The linearity of the calibration plots was confirmed by the high value of correlation coefficients ($r^2 = 0.9998 \pm 0.001$), and %RSD for the correlation coefficients was less than 2.

Accuracy

The accuracy of the method was ascertained by recovery method. When the method was used for extraction and subsequent analysis of the drug in the dosage form after

Table 3 Result of assay of TAP dosage form

Batch	Concentration of tablet samples (µg/ml)	Concentration found (µg/ml)	Percentage found
I	0.8	99.75	99.75
II	0.8	100.125	100.125
III	0.8	99.625	99.625
IV	0.8	99.875	99.875
V	0.8	100.125	100.125
VI	0.8	100.25	100.25
Mean		99.96	99.96
SD		0.246	0.246
%RSD		0.2459	0.2459

spiking with 50%, 100%, and 150% of the drug, the recovery was 100.05% (99.7% to 100.9%).

Precision

The repeatability of sample injection and measurement of peak area were expressed as %RSD (Table 2). Repeatability and intermediate precision at three different concentrations (0.4, 0.6, and 0.8 µg/ml) for both within-day and day-to-day analysis were always <2%. These low values of the %RSD showed that the repeatability and intermediate precision of the method were within the acceptable value.

Robustness

There was no significant change in the result of developed method, after the introduction of small deliberate changes in temperature ($\pm 5^\circ\text{C}$) and formic acid content in aqueous phase of the mobile phase ($\pm 5\%$). The standard deviation of peak areas was calculated for each set of conditions and was found to be <2% (Table 2). The low values of %RSD indicate that the method is robust.

LOQ and LOD

The developed method was highly sensitive to detect and determine the TAP content. The LOD for signal-to-noise ratio of 3:1 was 1 ng/ml (RSD \pm 0.067%), and the LOQ for signal-to-noise ratio of 10:1 was 8 ng/ml (RSD \pm 0.063%) (Table 2).

Specificity

A different set of condition for elution of the TAP was changed as discussed in previous sections, *viz.* formic acid content and temperature; then in spite of these changes, no additional peak was found, although a very small change was observed in retention times and peak shapes. The specificity of the method was ascertained by analyzing drug standard solution and samples of equivalent concentration (0.8 µg/ml). The identity of the peak in the sample was confirmed by comparison of the retention time and UV spectrum of the peak from the sample with those of the peak from the standard. Peak purity for the drug was assessed by comparing the UV spectra acquired at the peak start, peak apex, and peak end. The specificity of the method was also ascertained by analyzing the alkaline degraded samples; when degraded sample was chromatographed, the concentration of the TAP was reduced and other peaks were observed at different retention time.

Sample solution stability

The response ratio of all samples was averaged as 184.16 (Table 2). The standard deviation and percentage of relative standard deviation were found to be less than 2: 1.83 and 0.057, respectively.

Analysis of dosage form

TAP is freely water soluble, so drug content from tablet powder was extracted by triple distilled water. Sonication of drug powder with triple distilled water has better extraction compared to the shaking of solution. TAP content was determined between 99.79% and 100.33% with a standard deviation of 0.217. Error of standard deviation was far less than the unit which was favorable in consistency with the result of the method.

Conclusions

Hence, the new liquid chromatographic method in reverse phase was developed and validated; (Table 3) it was simple, fast, precise, and accurate without having to use diethylamine in the mobile phase and with UV detection. The method was successfully applied to determine the TAP content in tablet dosage form within acceptable limits and may be applied for assay, dissolution studies, bio-equivalence studies, as well as routine analysis in pharmaceutical industries.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Experimental work was done by PKB. Both authors equally contributed in experimental design, framing, writing, proofing and approval of the manuscript.

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