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Research Article

SIMULTANEOUS DETERMINATION OF TOLPERISONE AND PARACETAMOL IN PURE AND FIXED DOSE COMBINATION BY UV – SPECTROPHOTOMETRY

G.ABIRAMI* T.VETRICHELVAN[1]

Departmaent of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur. Email:abiramiganesan78@gmail.com

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ABSTRACT

Two new simple accurate and sensitive UV– spectrophotometeric methods have been developed for determination Simultaneous equation and Derivative spectroscopy method for Tolperisone and Paracetamol in bulk and in combined dosage form. Double distilled was used as a solvent. The wavelength selected for Simultaneous method for Tolperisone at 261nm and Paracetamol at 243nm respectively. Beer's law was obeyed with the concentration ranges from 0-2.5µg/ml, 3- 9µg/ml respectively. For derivative spectroscopy method(1-5µg/ml,6-30µg/ml) the zero order spectrum was derivatized to first order derivative with the zero crossing points of Tolperisone has maxima at 243nm and Paracetamol has maxima at 261nm.The % recovery was found in the range 99.0 ± 0.012, 100 ± 0.342.The developed method was validated statically by recovery studies. The %R.S.D was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid, accurate and successfully applied for simultaneous determination.

Keywords: Tolperisone (TPE), Paracetamol (PCL), Simultaneous equation, Derivative spectroscopy method.

INTRODUCTION

Tolperisone (TPE) a Piper dine derivative was a centrally-acting muscle relaxant. Typically, TPE is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurological diseases. Its IUPAC name was 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one with the Molecular formula $C_{16}H_{23}NO$. Paracetamol (PCL) is chemically N (hydroxyl phenyl acetamide) with the Molecular formula $C_8H_9NO_2$. It is used mainly used as antipyretic, a non- opioid and non-salicylate analgesic [4, 5] It is indicated for the treatment of moderate to severe pain. Paracetamol was official in Indian Pharmacopoeia [3] and British Pharmacopoeia. Both the drugs are available in combined tablet dosage form.

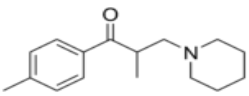

Fig. 1: Chemical structure of Tolperisone

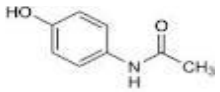

Fig. 2: Chemical structure of Paracetamol

Literature survey reveals that there are UV[6], HPLC[7-11] Capillary electrophoresis[12] methods were reported for the estimation of PCL and for TPE UV[19,20] HPLC methods [17,18] in pharmaceutical formulations in some other combinations and also in single dosage form. The extensive review of the literature revealed that no method was yet reported for the simultaneous estimation of both the drugs in combined dosage forms. This paper describes simple, rapid, accurate, reproducible and economical method for the Simultaneous estimation of TPE and PCL in tablet formulations using derivative method.

MATERIALS AND METHOD

Instrumentation

The present work was carried out on Shimadzu-1700 double beam UV visible spectrophotometer with a pair of 10mm matched quartz cell. Glass wares used were of A grade and soaked overnight in a mixture of chromic acid and sulfuric acid rinsed thoroughly with distilled water and dried in hot air oven. Shimadzu AUX- 200 digital balance.

Reagents and Chemicals

All the chemicals used were of analytical grade and procured from Qualigens, India Ltd. Distilled water of Analytical grade.TPE and PCL were procured as a gift sample from Amaranth pharmaceuticals, Pondicherry. Formulation purchased from Local pharmacy market.

MATERIALS AND METHODS

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility was carried out in polar to non polar solvents. The common solvent was found to be distilled water, used for the analysis of both TPE and PCL for the proposed method.

Preparation of standard stock solution

10 mg of TPE and PCL raw material were weighed and transferred into 10 ml volumetric flasks separately and dissolved in distilled water and made up to the volume with water. These solutions were observed to contain 1000 µg mL^{-1}.And further dilution was made to get concentration of 10 µg mL^{-1}

Selection of wavelength for Estimation

From the stock solutions of TPE and PCL, 10µg mL^{-1} concentration solutions were prepared .The stock solutions were scanned between the wavelength ranges from 200 - 400 nm by using distilled water as blank and the spectra were recorded. From the overlain spectra of TPE and PCL 261nm and 243 nm were selected for the estimation of by Simultaneous equation method. (Fig-3) For Derivative spectroscopy method, the zero order spectra was derivatized to first order derivative spectrum in that 261 nm was selected for the estimation of PCL, (zero crossing for TPE) and 243 nm was selected for the estimation of TPE (zero crossing for PCL) (Fig-4). The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that PCL was stable for 3 hours and TPE was stable for more than 3 days at all the selected wavelengths.

Spectral and Linearity Characteristics

The aliquots of stock solution of TPE (0.5-2.5ml of10 µg/ml) and PCL (0.5-2.5ml 0f 60µg/ml) were transferred into 10 ml volumetric flasks to get the concentration of (0.5-2.5µg/ml, 3- 9µg/ml) were made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 261, 243, nm in the normal spectrum for simultaneous equation method. The zero order spectra was derivatized to first order derivative spectra with the wavelengths 243 nm, 261 nm (1-5µg/ml, 6-30µg/ml)(zero crossing points for PCL and TPE respectively. The calibration curve was plotted at their corresponding wavelengths. All two drugs TPE

and PCL were found linear with the concentration range of 0.5-2.5 μg/ml and 3-15 μg/ml respectively at their respective wavelengths.

Analysis of marketed formulation

Twenty tablets of formulation (TPE 50 mg and 300 mg of PCL) were weighed accurately. The average weight of tablets were found and powdered. The tablet powder equivalent to 15 mg of TPE was weighed and transferred into 100 ml volumetric flask added a minimum quantity of distilled water to dissolved the substance by using ultra sonication for 15 minutes and made up to the volume with the same (1000 μg mL^{-1}). The content was filtered through whatman filter paper No. 41. From the cleared solution, further dilutions were made by diluting 1 ml to 10ml volumetric flask, further diluted 1 ml to 10 ml to obtain 1.5 μg mL^{-1} of TPE which contains 9μg mL^{-1} of PCL theoretically. The absorbance measurements were made 6 times for the formulation at 261 nm, 243 nm, in normal spectrum and 243nm and 261 nm. For the first order derivative spectrum, 2ml 0f 1.5μg mL^{-1} into 10ml standard flask contains 3μg mL^{-1} of TPE and18μg mL^{-1} of PCL theorectically.From the absorptivity values of TPE and PCL at 261 nm, 243 nm, the amount of TPE and PCL were determined by using Simultaneous equation method and Derivative spectroscopic method.

Method A: Simultaneous Equation Method

From the standard preparation, various dilutions were made at concentration range from 0.5-2.5μg/ml and 3-15μg/ml. It was observed that it obeys the Beer's law.

The simultaneous equations formed were,

At λ_1 $A_1 = ax_1bc_x + ay_1bc_y$ ------ (1)

A1 = 628 C_X + 376 C_Y ------ (2)

At λ_2 $A_2 = ax_2bc_x + ay_2bc_y$ ------- (3)

A2 = 288C_X + 730C_Y ------ (4)

Were A1 and A_2 are the absorbance of sample solution at 261 and 243 nm respectively. Cx and C_Y are the concentration of TPE and PCL respectively (μg /ml) in sample solution.

The absorbance's (A_{1}& A_2) of the sample solution were recorded at 261 and 243nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4). Precision of the method was determined by carrying out Intra-Day (n = 3) and Inter- Day (n = 3) studies.

Method B: Derivative Spectroscopy Determination

UV spectra of both the drugs (TPE and PCL) were derivatized to first order derivative with Δλ = 1 for the entire spectrum. Zero crossing points for TPE and PCL were found to be 261nm and 243 nm respectively (Fig 4). From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 1-6 μg/ml of TPE and 6-30 μg/ml of PCL and the readings were taken in the first order mode at the selected wavelengths. Optical and regression data were calculated. Accuracy of the method was checked by preparing five mixed standards containing different concentration, absorbance's were measured at respective zero crossing points in first order UV spectrum and amount present in the sample was calculated from their respective calibration curve. Precision of the method was determined by performing Intra -Day (n = 3) and Inter- Day (n = 3).

Recovery studies

The recovery experiment was done by adding known concentrations of TPE and PCL raw material to the 50% pre-analyzed formulation. Standard TPE and PCL in the range of 80 %, 100 % and 120% are added to the 50% pre-analyzed formulation into a series of 10 ml volumetric flasks , dissolved with distilled water and made up to the mark with the same. The contents were sonicated for 15minutes. After sonication the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for determination of TPE and PCL respectively. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous Equation method, and Derivative spectroscopic method. The procedure was repeated for three times

Validation of developed method

Linearity

A calibration curve was plotted as concentration vs. absorbance. TPE was found to be linear in the concentration range of 0.5 to 2.5 μg/ ml at 261 nm, PCL was found to be linear in the concentration range of 3 to 15 μg/ ml at 243 nm.

Precision

The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days, respectively. The amount of drugs was determined and % RSD was also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation performed in different instrument and also by the different analysts. The amount and % RSD were calculated.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation, known quantities of raw materials of TPE and PCL were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated found to be 0.994798

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept).

RESULTS AND DISCUSSION

A simple accurate and precise simultaneous equation method was developed and validated. The drug samples were identified by melting point check and IR spectrum. The solubility of PCL and TPE were determined as per I.P specifications. Trials were made with a variety of polar and non-polar solvents. From the solubility profile double distilled water were the common solvents for both the drugs.

10 μg/ ml concentrations of these two drugs were scanned in the UV region and the spectra were recorded. From the spectra the λ max of the drugs were found to be 261 nm for TPE, 243 nm for PCL. The zero order spectra were derivatized to first order derivative spectra with zero crossing at 261nm for TPE and 243nm zero crossing for PCL respectively. The spectra for TPE and PCL are shown in figure 3 and 4 respectively. .

The stability of the drugs was studied by measuring the absorbances at different time intervals. All the drugs are stable more than 3 hrs in distilled water.. Various aliquots of TPE & PCL in water were prepared for both simultaneous and derivative method in the concentration range of 0.5 – 2.5 μg/ ml, 3 – 15 μg/ ml respectively and the absorbance of those solutions were measured. The calibration curve was constructed. The preparation of calibration curve was repeated in six times for each drug at their selected wavelengths. The optical parameters like, sand ell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD and LOQ were calculated. The correlation coefficient for both drugs was found to be above 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The calibration curve was plotted using concentration against absorbance. To confirm the precision of the method, the analysis of formulation was repeated in six times. The

amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0 .994798, 0.130619 and 0.6679 for TPE and PCL respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in table 3.

The intermediate precision of the method was confirmed by intraday and inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time on three consecutive days. The % RSD value of intraday and inter-day analysis was found to be 0.37990 and 0.23915 for TPE, 0.915402 and 0.647017 for PCL. The results showed that the less % RSD value and it were confirmed that the intermediate precision of the method was good.

The developed method was also validated for ruggedness. It refers to the specificity of one lab to multiple days which may include different analysts, different instruments and different sources of reagents and so on. In the present work, it was confirmed by different analysts and by different instruments. The low % RSD values indicate that the developed method was more rugged. The results were shown in table

The accuracy of the method was confirmed by recovery studies. To the pre analyzed formulation, a known quantity of TPE and PCL raw material were added at different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98.98 – 100.77% for TPE, 99.28 to 100.45% for PCL. The low % RSD value for three drugs indicates that this method is very accurate. The recovery data's were shown in table 3. The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to be accurate.

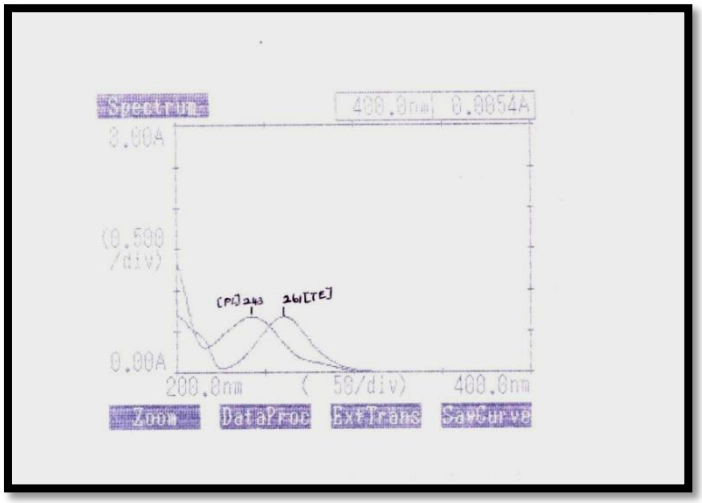

Fig. 3: Overlain Spectrum of TPE and PCL

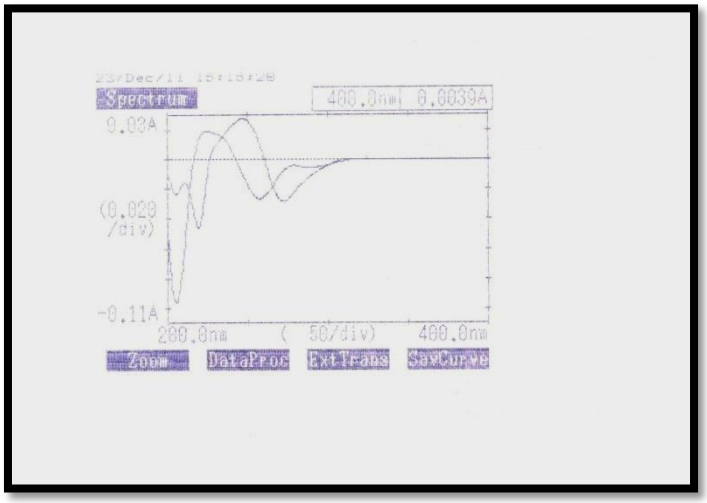

Fig. 4: Overlain Spectrum of Derivative Spectroscopy

Table 1: Optical Characteristics

Parameters	Method A		Method B			
	TPE	PCL	TPE	PCL	TPE	PCL
λ max (nm)	261nm	243nm	261nm	243nm	243nm	261nm
Beer's law limi	0.5-2.5	0.5-2.5	3-15	3-15	1-6	6-30
Sandell's sensitivity)	0.01587877	0.035098275	0.02717321	0.0137006	0.45632338	0.39407018
Molar absorptivity	17698.96	8116.41	5683.616	11034.68	129.6372	384.05283
Correlation coefficient (r)	0.99984	0.999638	0.9998	0.9995	0.99912	0.99965
Slope(m)	0.62977	0.0284914	0.036800952	0.072992	0.00219	0.00253
Intercept (c)	0.00010476	0.0001523	0.001942	0.00059047	0.0001047	1.9047
Standard error	0.00036436	0.00253358	0.0042700	0.001411	0.45632	0.0001385

Table 2: Quantification of tablet formulation

Drug		Label Claim	Amount Found	S.D	R.S.D	S.E
MET A	TPE	50mg	49.9mg	0.983192	0.994798	0.027311
	PCL	300mg	299.9mg	0.13084	0.130619	0.005215
MET B	TPE	50mg	49.86mg	0.07071	0.070534	0.17675
	PCL	300mg	298.9mg	0296985	0.299259	0.017873

Table 3: Recovery Studies

Methods		%	Amount Present* (µG ML^{-1})	Amount Added* (µG ML^{-1})	Amount Estimated* (µG ML^{-1})	Amount Recovered* (µG ML^{-1})	% Recovery*	S.D.	% R.S.D.	S.E.
MET A	TPE	80	1.5	1.2	2.7	2.699	99.60	0.36501	0.37018	0.04056
		100	1.5	1.5	3.0	4.5112	98.58	0.60929	0.61802	0.00677
		120	1.5	1.8	3.3	5.019	99.06	0.37207	0.37558	0.04134
	PCL	80	9	7.2	16.2	15.990	100.92	1.15725	1.14663	0.12858
		100	9	9	18	18.0109	101.57	1.90616	1.86927	0.2118
		120	9	10.8	19.8	19.601	99.49	1.28204	1.28004	0.1424
MET B	TPE	80	3	2.4	5.4	5.3961	99.8	0.378153	0.03799	0.01215
		100	3	3	6	6.001	100.01	0.238747	0.23915	0.00955
		120	3	3.6	9.6	9.5962	99.06	0.909776	0.91543	0.0.252
	PCL	80	18	14.4	32.4	32.145	100.92	0.0112	0.1005	0..0241
		100	18	18	36	35.9969	99.86	0.003714	0.3689	0.3265
		120	18	21.6	39.6	38.8962	98.42	0.00123	0.1526	0.1.002

Table 4: Intermediate precision and rugedness of method

Parameters	Label claim Estimated (method-a) TPE PCL	Label claim Estimated (method-b) TPE PCL
Intra day	100.01 100.05	99.5 100.01
Interday	101.02 99.26	98.23 99.52
Instrument -1	99.82 97.41	98.74 100.30
Instrument -1	98.76 101.25	99.99 98.52
Analyst-1	98.65 100.36	100.21 99.65
Analyst-1	100.54 99.85	100.10 98.71

CONCLUSION

All the above methods do not suffer from any interference due to common excipients. It indicates that methods were accurate. Therefore the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing TPE and PCL.

Thus the above study's findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms.

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REERENCES

1. Text on validation of Analytical procedures, ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 27, **1994**, 1-5.
2. Validation of Analytical procedures; Methodology, ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, **1996**, 1-8.
3. The Indian Pharmacopoeia. The Controller of Publication, Ghaziabad, Vol.II, **2007**, 701.
4. The Merck Index. 14th edn, Merck Research Laboratories Division of Merck & Co. Inc, USA, **2006**, 4389(756), 386(66).
5. The United States Pharmacopoeia, 22nd Revision United States Pharmacopoeia Convention, Inc., Rock villa, MC. USA, **1995**, 1776-1777.
6. Beckett, A.H. and Stenlake, J.B. Practical pharmaceutical chemistry. 4th Edition- part II, CBS Publishers and Distributors, New Delhi, **2007**, 278-300, 307-312.
7. Chatwal, R. Gurdeep. And Sham K. Anand. Instrumental Methods of Chemical Analysis, 5th revised edition, Himalaya Publication House, Mumbai, **2008**, 2.107 – 2.120.
8. Devala rao, G. A textbook of pharmaceutical analysis, 1st edn., vol II, Birla publications pvt Ltd, **2004**, 1.
9. Wadher SJ, Pathankar PR, Manisha Puranik, Ganjiwale RO, Yeole PG: Simultaneous spectrophotometric estimation of Paracetamol and Metoclopromide HCl in solid dosage form. Indian J of PharmSci **2008,** 70 (3): 393-395.
10. Ghada M, Hadad, Samy Emara, Waleed, Mahmand MM: Stability indicating RP-HPLC method for determination of Paracetamol with dantrolene and Cetrizine and Pseudoephedrine in two pharmaceutical dosage forms. Talanta **2009**, 79: 1360-1367.
11. Udupa N, Karthik A, Subramanian G, Ranjith Kumar A: Simultaneous estimation of Paracetamol and Domperidone by HPLC method. Indian J Pharm Sci **2007**, 69 (1), 140-144.
12. Subramanian G, Vasudevan M, Ravishankar S, Suresh B: Validation of RP-HPLC method for simultaneous determination of Paracetamol, Methocarbamol, Diclofenac potassium in tablets. Indian J Pharm Sci **2005,** 67 (2): 260-263.
13. Fijalek Z, Wyszecka-Kaszuba E, Warowna-Grzeskiewicz M: HPLC with amperometric detection for the determination of 4-aminophenol, the main impurity of Paracetamol in multicomponent analgesic preparation. J Pharm Biomed Anal **2003,** 32: 1081-1086.
14. Lotfi Monser, Frida Darghouth: Simultaneous LC determination of Paracetamol and related compound in pharmaceutical formulation using carbon based column. J of Pharm Biomed Anal. **2002,** 27.
15. Shulin Zahao, Dan Xiao, Wenling Bai, Hongyan Yuan: Capillary electrophoresis with chemiluminescence detection of Paracetamol. Anal Chim Acta **2006**, 559: 195-199.
16. Hofer D, Lohberger B, Steinecker B, Schmidt K, Quasthoff S, Schreibmayer W

17. (**2006)"***EurJPharmacol* **538** (13):.doi:10.1016/j.ejphar.2006.03.034. PMID 16650844
18. Jung-Woo Bae, Young-Seo Park, Uy-Dong Sohn, Chang-Sun Myung, Byung-Kwon Ryu, Choon- Gon Jang, and Seok-Yong Lee, Arch Pharm Res Vol 29, No 4, 339-342
19. Napaporn Youngvises, Boonsom Liawruangrath and Saisunee Liawruangrath, Journal of Chromatography.
20. R.Prashanthi, V.Jagathi, M.Shaiba, K.Raghavi, M.Sindhura, IJPI's Journal of Analytical Chemistry ISSN 2229 – 6867,**2011**
21. Mit J Patel, R Badmanaban, CN Patel Pharmaceutical methods : Vol : 2 | Issue : 2 | 124-129 **2011**
22. R.Prashanthi, V.Jagathi, M.Shaiba, K.Raghavi, M.Sindhura Asian journal of chemistry Volume 04, Issue 02, 2011