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Development and Validation of Uv-Spectrophotometric Method for Simultaneous Estimation of Naproxen and Paracetamol By Q-Absorbance Ratio Method.

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Abstract

A simple Q-absorbance ratio UV-spectrophotometric method was developed for the simultaneous estimation of Naproxen and Paracetamol in pharmaceutical dosage form. The solvent used was 0.1N NaOH. Two wavelengths 257.00 nm (λ_{max} of Paracetamol) and 234.00 nm (Isoabsorptive point) were selected for estimation of paracetamol and naproxen for Q-Absorbance ratio method. The overlay spectrum of both drugs was used to decide the isoabsorptive point for analysis. The linearity ranges for Paracetamol (PCM) and Naproxen (NPX) were 2.5 – 5.0 µg/ml and 1.5 – 3.0 µg/ml respectively. The % recovery was found to be 97.91% and 98.64% for paracetamol and naproxen respectively indicating that the proposed method was accurate and precise for simultaneous estimation of naproxen and paracetamol in bulk formulations.

Keywords: *Naproxen, Paracetamol, Q-absorbance ratio method, isoabsorptive point*

Introduction:

Paracetamol is chemically N-(4-hydroxyphenyl) acetamide. It has analgesic and antipyretic activity. Chemically naproxen is (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid. Naproxen has analgesic and antipyretic properties[1]. It works by inhibiting both the cox-1 and cox-2 enzymes. Naproxen was originally marketed as the prescription drug. It is also associated with the smallest overall cardiovascular risks [2]. Literature survey reveals that various HPLC [3], HPTLC [4], TLC/UV [5], LC-MS/MS [6] methods reported for the estimation of Naproxen alone in blood serum and in ocular fluids. Various methods are also available for estimation of paracetamol alone or in combination with other drugs [7,8,9]. However there is no simple and accurate method reported for the determination of Naproxen and Paracetamol in combined pharmaceutical formulation by UV-Visible spectrophotometry.

Materials and Methods:

Instrumentation:

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV-Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1 cm quartz cells over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A×120, shimadzu).

Reagents and Chemicals:

Distilled water, NaOH pellets (LOBA Chemie Pvt. Ltd., Mumbai, India) Paracetamol and Naproxen was obtained as a gift sample from Molecular laboratories, Ahmedabad

Preparation of Sodium Hydroxide (0.1N)
About 4.0gm of Sodium Hydroxide was dissolved in 1000ml of distilled water.

Preparation of Standard stock solution of Paracetamol (Solution A, 50 µg/ml)
An accurately weighed quantity of about 5 mg of Paracetamol was weighed and transferred in a 100 ml volumetric flask. About 70 ml of 0.1N NaOH was added to it and sonicated. The volume was made upto the mark with 0.1N NaOH

Preparation of working standard solution of Paracetamol (Solution B, 5 µg/ml)
About 1 ml of Solution A was diluted to 10 ml with 0.1N NaOH.

Preparation of standard stock solution of Naproxen (Solution C, 30 µg/ml)
An accurately weighed quantity of about 3 mg of Paracetamol was weighed and transferred in a 100 ml volumetric flask. About 70 ml of 0.1N NaOH was added to it and sonicated. The volume was made upto the mark with 0.1N NaOH

Preparation of working standard solution of Naproxen (Solution D, 3 µg/ml)
About 1 ml of Solution C was diluted to 10 ml with 0.1N NaOH.

Selection of Wavelength for analysis:

Working Standard solutions (Solution B and D) were separately scanned in the range of 400-200nm. The spectra of PCM and NPX were recorded. The overlay spectrum was used to decide the isoabsorptive point for analysis.
Two Wavelengths 257.00 nm (λ_{max} of Paracetamol) and 234.00 nm (Iso-absorptive point) were selected for estimation of PCM and NPX for Q-Absorbance ratio method.

Study of Beer-Lambert Law:

Aliquots of solution A and of solution B (0.5-1.0 ml) were transferred separately into five 10.0 ml volumetric flask and diluted upto the mark with 0.1 N NaOH to obtain the final concentration in the range of 2.5 - 5 µg/ml of PCM and 1.5 – 3 µg/ml of NPX. Absorbance of the solutions were recorded at 257 nm (λ_{max} of Paracetamol) and 235 nm (λ_{max} of Naproxen). The Calibration graph of Concentration Vs Absorbance was plotted to determine the linearity and range of analytical method.

The observations and results of linearity study are shown in table I

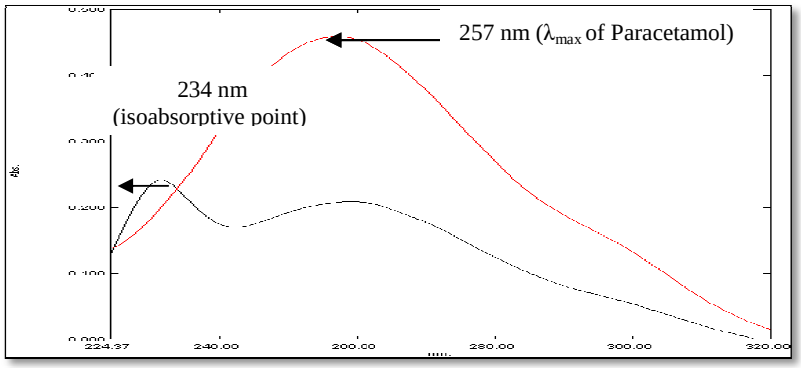

Figure 1: Overlay spectrum of naproxen and paracetamol

Table I: Results of Linearity

Sr. No.	Concentration (µg/ml)		Absorbance	
	PCM	NPX	PCM (257 nm)	NPX (235 nm)
01	2.5	1.5	0.234	0.115
02	3.0	1.8	0.276	0.138
03	3.5	2.1	0.320	0.163
04	4.0	2.4	0.367	0.188
05	4.5	2.7	0.407	0.215
06	5.0	3.0	0.452	0.240

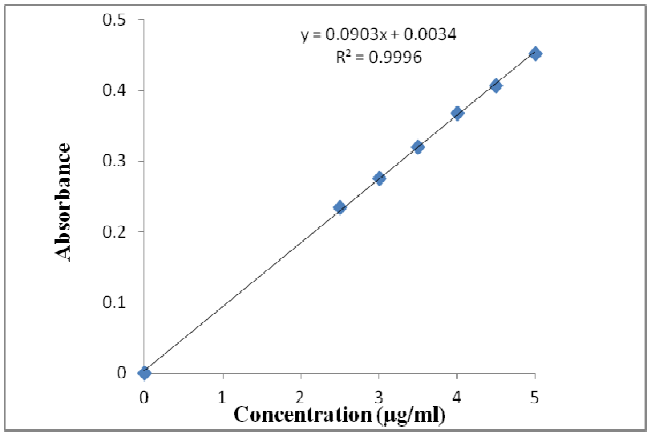

Figure 3: Linearity of PCM

PCM was found to be linear over the concentration range of 2.5 – 5 µg/ml.

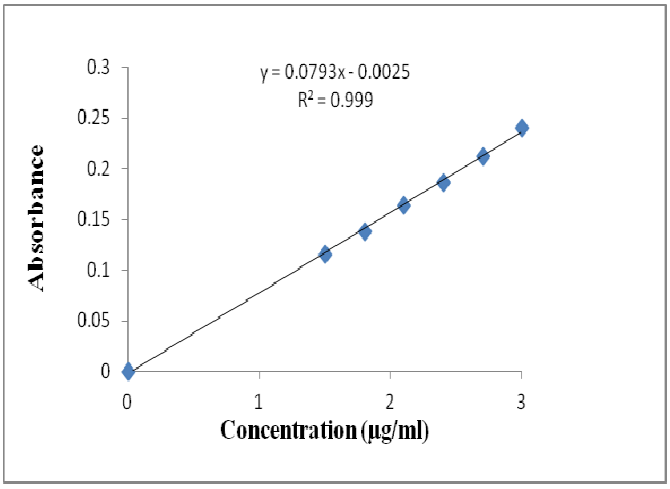

Figure 4: Linearity of NPX

NPX was found to be linear over the concentration range of 1.5 – 3 µg/ml.

Application of the proposed method to the physical mixture:

Sample Solution:
An accurately weighed quantity of 5 mg of PCM and 3 mg of NPX was transferred into a 100 ml of volumetric flask. About 70 ml of 0.1N NaOH was added to it and sonicated. The volume was made upto the mark with 0.1N NaOH.

Procedure:
Aliquots of 0.5 ml of above solution was transferred into a 10.0 ml of volumetric flask and diluted upto the mark with 0.1 N NaOH to obtain final concentration of 2.5 µg/ml for PCM and 1.5 µg/ml for NPX. Absorbances of the solutions were recorded at 257 nm (λ_{max} of Paracetamol) and 234 nm (Iso-absorptive point).

Calculation:
Concentration of both the drugs was determined using the formula mentioned below:

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a}$$

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A}{a}$$

Where,
C_1= Concentration of Paracetamol (µg/ml)
C_2= Concentration of Naproxen (µg/ml)
A = Absorbance of sample at Iso-absorptive point (234 nm)
a = Absorptivity of Paracetamol (0.0439095) and Naproxen (0.07498104) at iso-absorptive point
Q_1= Ratio of absorbance of Paracetamol at 257 nm to absorbance of Paracetamol at 234 nm
(0.452/0.214 = 2.112)
Q_2 = Ratio of absorbance of Naproxen at 257 nm to absorbance of Naproxen at 234 nm
(0.207/0.228 = 0.907894)
Q_0 = Ratio of absorbance of sample at 257 nm to absorbance of sample at 234 nm

Result:
The results of analysis of physical mixture are shown in table II

Table II: Results of analysis of physical mixture

Sr. No.	Weight of Standa (mg)		Amount tak (µg/ml)		Absorbance		Amount fou (µg/ml)		% Purity	
	PCM	NPX	PCM	NPX	234 nm	257 nm	PCM	NPX	PCM	NPX
01	5	3	2.5	1.5	0.219	0.326	2.41	1.51	96.21	100.81
02	5	3	2.5	1.5	0.218	0.325	2.40	1.49	96.14	99.99
03	5	3	2.5	1.5	0.219	0.326	2.41	1.51	96.21	100.80
04	5	3	2.5	1.5	0.218	0.325	2.40	1.49	96.14	99.99
05	5	3	2.5	1.5	0.220	0.328	2.43	1.51	97.04	100.89
06	5	3	2.5	1.5	0.216	0.322	2.38	1.49	96.07	99.17
								Mean	96.30	100.28
								SD	0.37	0.68
								RSD(%)	0.38	0.67

The percentage purity was found to be 96.07 for PCM and 100.28 for NPX.

Validation of the proposed method:
The proposed method was validated as per ICH Q2 (R1) guidelines for various parameters like accuracy, precision, linearity and range, limit of detection, limit of quantitation etc.

Accuracy:
The accuracy of the proposed method was determined by the recovery study. The known amount of pure drug is spiked into the pre-analyzed synthetic mixture. Analysis was carried out at three concentration levels such as 80%, 100% and 120% within the specified linearity range.

Sample solution:
An accurately weighed quantity of about 5.0 mg of PCM and 3.0 mg of NPX was transferred into 100.0 ml volumetric flask. About 70 ml of 0.1N NaOH was added to it and sonicated. The volume was made upto the mark with 0.1 N NaOH. The solution was filtered through 0.25µ nylon filter. About 0.5 ml of above solution was transferred into six 10 ml volumetric flask and 0.4 ml, 0.5 ml and 0.6 ml of standard stock solution of PCM (Solution A) and standard stock solution of NPX (Solution C) was added into the respective volumetric flask, and final volume was made upto the mark with 0.1N NaOH.

Procedure:
Absorbance of the solutions were recorded at 257 nm (λ_{max} of Paracetamol) and 234 nm (Iso-absorptive point).

Calculation:
The content of mixture of PCM and NPX was calculated using equations mentioned in calculation section.

The percentage recovery was calculated using the formula mentioned below:

$$\% \text{ recovery} = \frac{\text{Amount of drug found}}{\text{Amount of drug added}} \times 100$$

The results of Recovery study are shown in table III

Table III: Results of recovery study

Sr. No.	Amount taken (µg/ml)		Amount add (µg/ml)		Absorbance		Amount fou (µg/ml)		% Recovery	
	PCM	**NPX**	**PCM**	**NPX**	**234 nm**	**257 nm**	**PCM**	**NPX**	**PCM**	**NPX**
80%	2.5	1.5	2	1.2	0.399	0.600	4.49	2.68	99.92	99.56
	2.5	1.5	2	1.2	0.401	0.601	4.48	2.73	99.58	100.87
	2.5	1.5	2	1.2	0.398	0.599	4.49	2.68	99.88	99.10
100%	2.5	1.5	2.5	1.5	0.433	0.648	4.82	2.95	96.40	98.33
	2.5	1.5	2.5	1.5	0.435	0.649	4.805	2.987	96.10	99.56
	2.5	1.5	2.5	1.5	0.433	0.648	4.82	2.95	96.40	98.33
120%	2.5	1.5	3	1.8	0.478	0.720	5.41	3.21	98.36	97.27
	2.5	1.5	3	1.8	0.477	0.710	5.24	3.29	95.3	99.69
	2.5	1.5	3	1.8	0.475	0.720	5.46	3.14	99.27	95.15
								Mean	97.91	98.64
								SD	1.74	1.66
								% RSD	1.76	1.68

The mean % recovery was found to be 99.27 for PCM and 98.64 for NPX.

Precision:

The precision study of the method was determined as repeatability and intermediate precision study. The repeatability study (inter- day precision) was performed by analyzing homogenous tablet sample of formulation. The intermediate precision study was performed by variation in days of analysis.

Sample solution:
An accurately weighed quantity of about 5.0 mg of PCM and 3.0 mg of NPX was transferred into 100.0 ml volumetric flask. About 70 ml of 0.1N NaOH was added to it and sonicated. The volume was made upto the mark with 0.1N NaOH. The solution was filtered through 0.25µ nylon filter. About 0.5 ml of above solution was transferred to 10 ml volumetric flask, and final volume was made upto the mark with 0.1N NaOH.

Similarly five more samples were prepared.

Procedure:
Absorbance of the solutions were recorded at 257 nm (λ_{max} of Paracetamol) and 234 nm (Iso-absorptive point).

Calculation:
The content of mixture of PCM and NPX was calculated using equations mentioned in calculation section.

The results were expressed as SD, %RSD as shown in table IV.

Table IV: Results of Precision

Sr. No.	Inter- day precision		Intra- day precision	
	PCM	**NPX**	**PCM**	**NPX**
01	96.21	100.81	97.88	101.64
02	96.14	99.99	97.21	99.35
03	96.21	100.80	97.19	99.89
04	96.14	99.99	98.59	101.18
05	97.04	100.89	97.15	99.20
06	96.07	99.17	97.21	99.35
Mean	96.30	100.28	97.54	100.1
SD	0.37	0.68	0.58	1.05
% RSD	0.38	0.67	0.59	1.05

The results of inter- day precision were expressed as % RSD and it was found to be 0.38 and 0.67 for PCM and NPX respectively. The results of intra- day precision were expressed as % RSD and it was found to be 0.59 and 1.05 for PCM and NPX respectively. The % RSD value indicates the good precision of developed method.

Linearity and range:

The calibration graph was plotted between concentration Vs absorbance and linear relationship was found in concentration range of 2.5 – 5 µg/ml for PCM (R^2 = 0.9996) at 257 nm (λ_{max} of Paracetamol) and 1.5 – 3 µg/ml for NPX (R^2 = 0.999) at 235 nm (λ_{max} of Naproxen).

Results and discussion:

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 234 nm (isoabsorptive point) and 257 nm (λ-max of PCM) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of naproxen (235 nm) and paracetamol (257 nm) showing iso-absorptive point (234 nm) in 0.1 N NaOH is shown in Figure 1. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (as shown in Table III). Precision was calculated as repeatability for both the drugs (as shown in Table IV). Hence, the method can be employed for the routine analysis of these two drugs in combined dosage form.

Conclusion:

The proposed absorption ratio method was found to be simple, sensitive and accurate for determination of naproxen and paracetamol in the tablet dosage form. In this method the solvent used is easily available and cheap for the analysis of naproxen and paracetamol hence, this method was also economic for estimation of tablet dosage form. The common excipients and other additives used in the formulation doesn't interfere in the analysis of the tablet dosage form. So, we can say that this method can easily be adopted for combined dosage form in the pharmaceutical preparations.

"Cite this article"

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