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Development and Validation of Absorbance Correction Method for Simultaneous Estimation of Paracetamol, Ibuprofen and Dextromethorphan

Rakesh D Parmar, Dr. Pankaj Kapupara and Dr. Ketan Shah*

Department of Quality Assurance, School of Pharmacy, RK University, Kasturbadham, Rajkot-360020, Gujarat, India

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ABSTRACT

In current study infrared spectroscopy was performed for identification of Paracetamol (PCM), Ibuprofen (IBU) and Dextromethorphan (DEX). Simultaneous spectrophotometric determination of Paracetamol, Ibuprofen and Dextromethorphan was carried out by absorbance correction method using UV visible double beam spectrophotometer. Validation of simultaneous spectrophotometric method was performed with different parameter like accuracy, precision, recovery and LOD and LOQ. For absorbance correction method 226 nm, 247 nm and 279 nm were selected as working wavelength because at 279 nm and 226 nm Dextromethorphan, at 226 nm Ibuprofen and at 226 nm, 247 nm 279 nm Paracetamol showed significant absorbance. Linearity of Paracetamol, Ibuprofen and Dextromethorphan was found to be 2-40 µg/mL, 2-50 µg/mL and 1-10 µg/mL respectively. The % mean, standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) were calculated. The % R.S.D. was less than 2% as required by USP and ICH guideline. Present study revealed that absorbance correction method was found to be simple, accurate and precise. The experimental work involved is very simple, it requires only measurement of absorbance at selected wavelength. Current study revealed development and validation of simple, accurate, precise, rapid, cost effective, reproducible and reliable UV spectrophotometric method for estimation of Paracetamol, Ibuprofen and Dextromethorphan in bulk and synthetic mixture.

Keywords: UV; Validation; Analytical method; Paracetamol; Ibuprofen; Dextromethorphan

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INTRODUCTION

Non-steroidal anti-Inflammatory drugs are medicine that relieves pain, swelling, stiffness and inflammation [1,2]. Non-steroidal anti-Inflammatory drugs also referred to as NSAID are the most common medicine prescribed for the treatment of illnesses such as arthritis. The majority of the individuals are familiar with the non-prescription NSAIDs including aspirin and ibuprofen that are sold over-the-counter [3]. The drug used for immediate symptomatic relief of cough are called antitussive agent. As a defensive mechanism, when any foreign material enters the respiratory tract the sensory impulse carry the message to the medulla where although cough has protective function [4]. UV spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products. Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies [5,6]. Ibuprofen is a Non-steroidal Anti-inflammatory agent (NSAID), analgesic (pain-killing) and antipyretic (fever-reducing) action. Chemically it is (RS)-2-(4-isobutylphenyl) Propionic acid.[7, 8] Dextromethorphan is chemically (+)-3-methoxy-9a-methylmorphian; (9S, 13S, 14S)-6, 18-Dideoxy 7, 8-Dihydro-3-O-methylmorphian. Dextromethorphan is a cough suppressant used for relief of non-productive cough [9,10].

MATERIALS AND METHODS

Paracetamol, Ibuprofen and Dextromethorphan

Preparation of standard stock solution [11]

10 mg each of Paracetamol, Ibuprofen and Dextromethorphan were weighed separately and transferred in three different 100 mL volumetric flask. All the drugs were dissolved in 50 mL of methanol with vigorous shaking and then volume was made up to the mark with methanol to obtain final concentration of 100 µg/mL of each solution of Paracetamol, Ibuprofen and Dextromethorphan.

Selection of analytical wavelength [11]

Using appropriate dilution of standard stock solution, the three solutions were scanned separately in order to get good result. By using overlay spectra of three drugs, working wavelength selected were 279 nm, 226 nm and 247 nm (5.2). Here, amount of Paracetamol was determined at 247 nm using standard calibration curve (using equations y=mx+c), where Paracetamol gives significant absorbance value. At 279 nm, Ibuprofen does not show absorbance, but Dextromethorphan and Paracetamol gives significant absorbance value. So, amount of Dextromethorphan was determined at 279 nm after correcting absorbance of Paracetamol. The concentration of Ibuprofen was determined at 226 nm after correcting absorbance of Paracetamol and Dextromethorphan.

Selection of analytical concentration range [11,12]

For each drug appropriate aliquots were pipette out from standard stock solution into series of 10 mL volumetric flask. The volume was made up to the mark with methanol to get a set of solution having the concentration of 1 to 10 µg/mL for Dextromethorphan and 2,4,6,8,10,15,20,25,30,35, 40, 45 and 50 µg/mL for Ibuprofen and 2, 4,6,8,10,15,20,25,30, 35 and 40 µg/mL for Paracetamol. The absorbance of Dextromethorphan was measured at 226 nm and 279 nm. The absorbance of Ibuprofen was measured at 226 nm; the absorbance of Paracetamol was measured at 226 nm, 247 nm and 279 nm. The absorbance was plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 1µg/mL to 10 µg/mL for Dextromethorphan, 2 µg/mL to 50 µg/mL for Ibuprofen and 2 µg/mL and 40 µg/mL for Paracetamol (Tables 1-5) (Figures 1-8).

Determination of absorptivity at analytical wavelength

The absorptivity of all three drugs was calculated at selected wavelength using data of calibration curve (Table 1). The absorptivity was used in forming equations for absorption correction method (equations 1, 2 and 3).

Table 1: Absorptivity measurement for absorbance correction method.

	Paracetamol	**Dextromethorphan**	**Ibuprofen**
A_1 (226)	431.20 (ax_1)	558.49 (ay_1)	245.75 (az_1)
A_2 (247)	957 (ax_2)	-	-
A_3 (279)	190 (ax_3)	229.10 (ay_3)	-

The concentration CPCM, CDEX and CIBU can be obtained by solving equation (equation no X, Y and Z) [12].

For Paracetamol

Cpcm (gm/100 mL)=(A2)/ax2
=(A2)/957 …… (1)

For Dextromethorphan

CDex (gm/100 mL)=(A3 – ax3 *CPcm)/ay3
=(A3 – 190 * CPcm)/229.10 …… (2)

For Ibuprofen

Cibu=[A1 – (ax1 * Cpcm + ay1 Cdex)]/az1
=[A1 – (431.20 * Cpcm +558.49]/245.75 ……(3)

Where,

A1=Absorbance of sample solution at 226 nm
A2=Absorbance of sample solution at 247 nm
A3=Absorbance of sample solution at 279 nm

IBU

(az1)=absorptivity Coefficients of IBU at 226 nm

PCM

(ax1)=absorptivity Coefficients of PCM at226 nm
(ax2)=absorptivity Coefficients of PCM at 247 nm
(ax3)=absorptivityCoefficientsof PCM at 279 nm

DEX

Ay1=absorptivity Coefficients of DEXat 226 nm
Ay3=absorptivity Coefficients of DEX at 279 nm
CIBU, concentration of IBU in g/100 mL in mixture
CPCM, concentration of PCM in g/100 mL in mixture
CDEX, concentration of DEX in g/100 mL in mixture
Now if a mixture of Paracetamol, Ibuprofen and Dextromethorphan were to be analyzed, a solution of suitable dilution should be prepared in solvent. The absorbance of the solution at 226 nm, 247 nm and 279 nm were measured. The values were substituted in equation (1, 2 and 3) to get a concentration of Paracetamol, Dextromethorphan and Ibuprofen.

Procedure for analysis of powder mixture

Mixed solution of pure drug was prepared by taking suitable volume of standard drug solution. Here, 0.3 mL standard solution of Dextromethorphan (100 µg/mL), 3 mL standard solution of Paracetamol (100 µg/mL) and 3.7 mL standardsolution of Ibuprofen (100 µg/mL) were transferred into 10 mL volumetric flask to make final concentration of 3 µg/mL for Dextromethorphan, 30 µg/mL for Paracetamol and 37 µg/mL for Ibuprofen. Absorbance of this prepared mixed solution was measured at 226, 247 and 279 nm (Figures 3-8) Concentration of Dextromethorphan, Ibuprofen and Paracetamol was calculated by putting absorbance values into equations 1, 2 and 3. Results of this analysis of powder mixture are reported (Tables 1-7).

Procedure for preparation of synthetic mixture

Synthetic mixture was prepared by adding various tablet ingredients to the drugs.

Table 2: Formulation of synthetic mixture.

Name of Ingredients	Quantity per Unit Dosage Form
Dextromethorphan	32.5 mg
Ibuprofen	400 mg
Paracetamol	325 mg
Lactose	157.5 mg
Talc	10 mg
Magnesium stearate	40 mg
PVP	10 mg
Total weight	975 mg

Procedure for analysis of synthetic mixture

Average weight of synthetic mixture for unit dosage form was found to be 975 mg. From the synthetic mixture, 30 mg powder (equivalent to 1 mg of Dextromethorphan, 10 mg of Paracetamol and 12.3 mg of Ibuprofen) was weighed and transferred into 100 mL volumetric flask and dissolved in methanol and the content was kept in ultra sonicator for 20 min. Finally the volume was made upto mark with methanol. The solution was filtrated through whatman filter paper number 41. From this solution 3 mL was transferred into another 10 ml volumetric flask. Volume was made upto the mark with methanol to make final concentration of 3 µg/mL for Dextromethorphan, 30

µg/mL for Paracetamol, 37 µg/mL or Ibuprofen. Absorbance of this prepared solution was measured at 226 nm, 247 nm and 279 nm. Concentration of Dextromethorphan, Paracetamol and Ibuprofen in synthetic mixture was calculated by putting absorbance values into equations 1, 2 and 3. Results of this analysis of synthetic mixture are reported in Table 8 and 9.

Procedure for recovery studies[13]

Recovery studies were carried out by applying the method to drug sample present in synthetic mixture to which known amount of Paracetamol, Ibuprofen and Dextromethorphan corresponding to 80,100,120% of label claim was added (standard addition method). In 80% recovery study, amount of standard added is 260 mg of Paracetamol, 296 mg Ibuprofen and 26 mg Dextromethorphan (i.e., 80 addition). In 100% recovery study, amount of standard added is 325 mg of Paracetamol and 400 mg Ibuprofen and 32.5 mg Dextromethorphan. (i.e., 100% addition). In 120% recovery study, amount of standard added is 390 mg of Paracetamol and 480 mg Ibuprofen and 39 mg Dextromethorphan (i.e., 120 addition). After the addition of the standard the synthetic mixture were mixed properly. From this tablets and standard mixture, mixed powder (equivalent to 10 mg Paracetamol, 12.30 mg Ibuprofen and 1 mg of Dextromethorphan was weighed and transferred into 100 mL volumetric flask and dissolved in methanol and content was kept in ultrasonicator for 20 min. Finally the volume was made upto the mark with methanol. The solution was filtered through whatman filter paper No. 41.
From this solution 3 mL was transferred into 10 mL volumetric flask and volume was made up to mark. Absorbance of this prepared solution was measured at 226 nm, 247 nm and 279 nm. Concentration of Paracetamol, Ibuprofen and Dextromethorphan was calculated by putting absorbance values into equation (1, 2 and 3)
The statistical evaluation data for recovery studies are shown in Table 10.

Procedure for precision [14]

Precision of the method was determined with synthetic mixture. Average weight of synthetic mixture for unit dosage form was found to be 975 mg. From the synthetic mixture, 30 mg tablets powder (equivalent to 1 mg of Dextromethorphan, 10 mg of Paracetamol and 12.3 mg of Ibuprofen) was weighed and transferred into 100 mL volumetric flask and dissolve in methanol and the content was kept in ultra sonicator for 20 min. Finally the volume was made upto mark with methanol. The solution was filtrated through whatman filter paper number 41.
This solution was further diluted as per the analysis of synthetic mixture to obtain mixed sample solution in Beer Lambert's range for each drug containing 30 µg/mL of Paracetamol, 37 µg/mL of Ibuprofen and 3 µg/mL of Dextromethorphan respectively. The mixed sample solutions were analyzed to obtain spectra and absorbance value at 226 nm, 247 nm and 279 nm were noted. The concentration of Paracetamol, Dextromethorphan and Ibuprofen were calculated from the equation. In intraday precision sample having concentration of 30 µg/mL of Paracetamol, 37 µg/mL of Ibuprofen and 3 µg/mL of Dextromethorphan was scanned six times at different time interval in the same day. Interday precision was obtained by the assay of six sample sets on different days as per the same procedure (Tables 11-13).

Determination of Limit of Detection and Limit of Quantitation

Limit of detection and limit of quantitation

Calibration curve was repeated six times and the standard deviation of the intercepts was calculated. Then LOD and LOQ were calculated as follow [15]:

$$\mathbf{LOD} = \frac{\mathbf{3.3*D}}{\mathbf{S}} \text{..... (4)}$$

$$\mathbf{LOQ} = \frac{\mathbf{10*D}}{\mathbf{S}} \text{..... (5)}$$

Where, D=Standard Deviation of y-intercepts of regression line of calibration curves.
S=Slope of the calibration curve
The result for LOD and LOQ are shown in Table 13.

RESULTS, DISCUSSION AND CONCLUSION

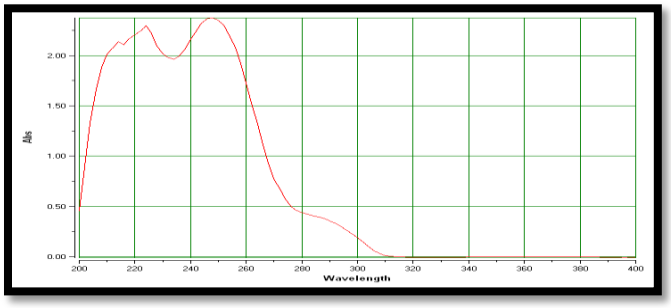

Figure 1: Mixture spectrum of Ibuprofen – 37 µg/mL, Dextromethorphan – 3 µg/mL and Paracetamol – 30 µg/mL in methanol

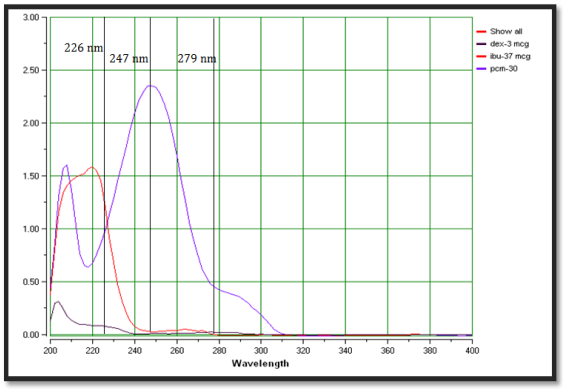

Figure 2: Overlay spectra of Ibuprofen – 37 µg/mL, Dextromethorphan – 3 µg/mL and Paracetamol – 30 µg/mL in methanol

From examination of overlay spectra of drugs, the three wavelength chosen were 226 nm, 247 nm and 279 nm. Here, Dextromethorphan showed absorbance at 226 nm and 279 nm, Paracetamol showed absorbance at 226 nm, 247 nm and 279 nm, Whereas Ibuprofen showed absorbance at 226 nm.

Selection of Analytical Concentration Range

Analytical concentration ranges for which drug obeys Beer Lambert's law were determined for all three drugs at selected wavelengths.

Table 3: Selection of analytical concentration range for Dextromethorphan (Linearity)

Sr. No.	Concentration (µg/mL)	Absorbance	
		226 nm	279 nm
1	1	0.045	0.016
2	2	0.086	0.036
3	3	0.151	0.056
4	4	0.221	0.075
5	5	0.276	0.108
6	6	0.341	0.131
7	7	0.401	0.158
8	8	0.461	0.19
9	9	0.511	0.221
10	10	0.573	0.265

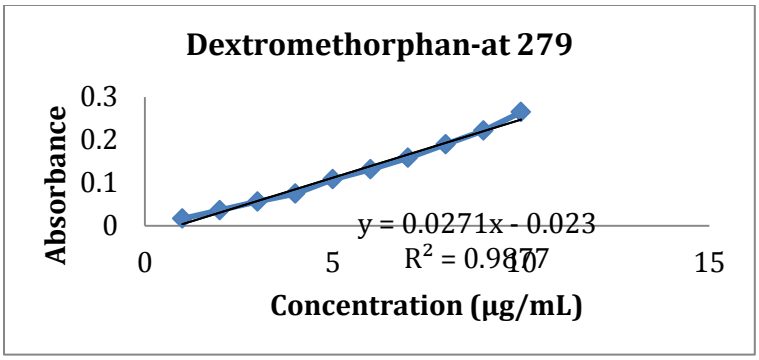

Figure 3: Calibration curve of Dextromethorphan at 279 nm

From the calibration curve of Dextromethorphan at 279 nm, the linearity was observed for 2 µg/mL to 10 µg/mL with correlation coefficient (r2) value of 0.987

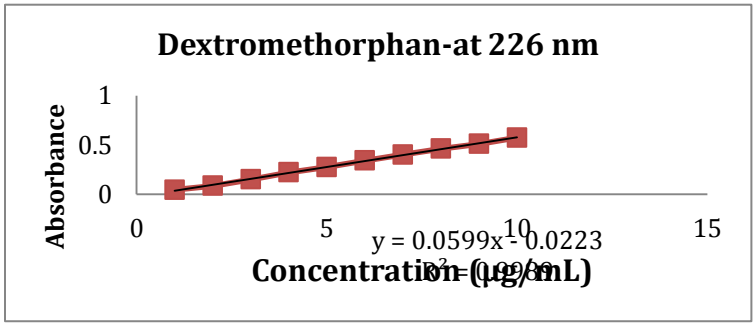

Figure 4: Calibration curve of Dextromethorphan at 226 nm

Table 4: Selection of analytical concentration range for Ibuprofen (Linearity)

Sr. No.	Concentration (µg/mL)	Absorbance 226 nm
1	2	0.05
2	4	0.1
3	6	0.128
4	8	0.198
5	10	0.28
6	15	0.393
8	20	0.476
9	25	0.578
10	30	0.771
11	35	0.861
12	40	0.981
13	45	1.1
14	50	1.2

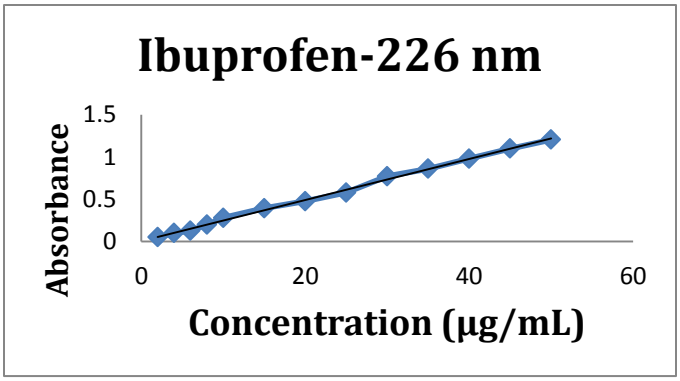

Figure 5: Calibration curve of Ibuprofen at 226 nm

From the calibration curve of Ibuprofen at 226 nm, the linearity was observed for 2 µg/mL to 50 µg/mL with correlation coefficient (r2) value of 0.997.

Table 5: Selection of analytical concentration range for Paracetamol (Linearity)

Sr. No.	Concentration	Absorbance		
	(µg/mL)	226 nm	247 nm	279 nm
1	2	0.081	0.193	0.038
2	4	0.165	0.395	0.073
3	6	0.276	0.59	0.113
4	8	0.346	0.79	0.153
5	10	0.415	0.931	0.213
6	15	0.645	1.501	0.286
8	20	0.816	1.921	0.35
9	25	1.121	2.396	0.481
10	30	1.245	2.835	0.581
11	35	1.581	3.305	0.663
12	40	1.713	3.801	0.75

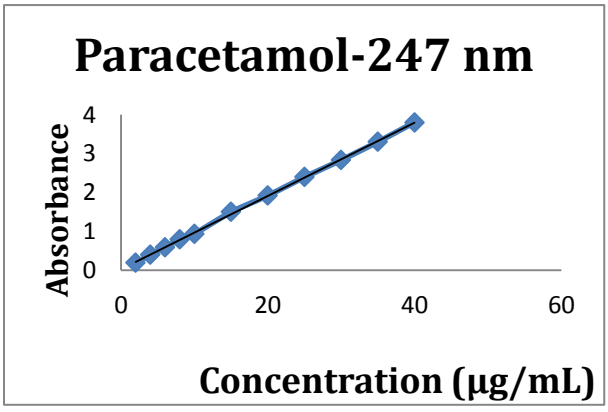

Figure 6: Calibration curve of Paracetamol at 247 nm

From the calibration curve of Paracetamol at 247 nm, the linearity was observed for 2 µg/mL to 40 µg/mL with correlation coefficient (r2) value of 0.999.

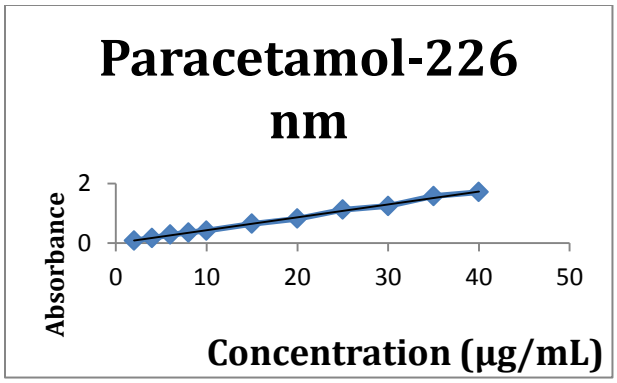

Figure 7: Calibration curve of Paracetamol at 226 nm

From the calibration curve of Paracetamol at 226 nm, the linearity was observed for 2 µg/mL to 40 µg/mL with correlation coefficient (r2) value of 0.996.

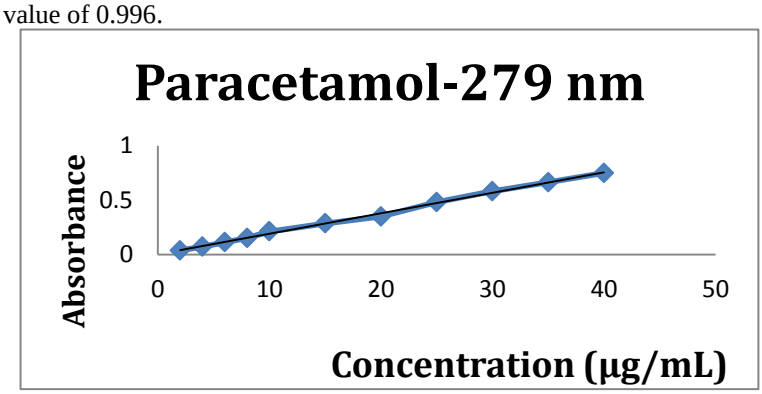

Figure 8: Calibration curve of Paracetamol at 279 nm

From the calibration curve of Paracetamol at 279 nm, the linearity was observed for 2 µg/mL to 40 µg/mL with correlation coefficient (r2) value of 0.997.

Table 6: Analysis of powder mixture

Sr. No.	Amount Present (µg/mL)			Amount Found (µg/mL)			Amount Found (%)		
	DEX	IBU	PCM	DEX	IBU	PCM	DEX	IBU	PCM
1	3	37	30	3	36.18	29.43	100.13	98.05	99.1
2	3	37	30	2.95	36.24	29.44	98.39	98.21	98.13
3	3	37	30	3.01	36.22	29.41	100.42	98.16	98.06
4	3	37	30	2.94	36.25	29.44	101.3	98.23	98.13
5	3	37	30	3.06	36.27	29.47	101.89	98.3	98.23
6	3	37	30	2.97	36.69	29.41	98.97	99.42	98.06

Table 7: Statistical validation for powder mixture

Drug	Mean* %	Standard Deviation	Co–efficient of Variation* (% R.S.D)	Standard Error*
DEX	100.18	1.335	1.332	0.544
IBU	98.39	0.508	0.516	0.207
PCM	98.28	0.401	0.408	0.165

The % R.S.D. was found to be less than 2% as required by USP and ICH guideline.

Analysis of Synthetic Mixture

Synthetic mixture was prepared by adding various tablet ingredients to the drugs.

Table 8: Formulation for synthetic mixture

Name of Ingredients	Quantity per Unit Dosage Form
Dextromethorphan	32.5 mg
Ibuprofen	400 mg
Paracetamol	325 mg
Lactose	157.5 mg
Talc	10 mg
Magnesium stearate	40 mg
PVP	10 mg
Total weight	975 mg

Table 9: Analysis of synthetic mixture (SM)

Sr. No.	Label Claim (mg/SM)			Amount of Drug Present in Prepared Synthetic Mixture Solution (µg/mL)			Amount Found (µg/mL)			% Label Claim		
	DEX	IBU	PCM	DEX	IBU	PCM	DEX	IBU	PCM	DEX	IBU	PCM
1	32.5	400	325	3	37	30	2.95	36.3	29.7	98.4	98.6	99
2	32.5	400	325	3	37	30	2.95	36.4	29.7	98.4	98.8	99.1
3	32.5	400	325	3	37	30	2.95	36.5	29.8	98.4	99	99.3
4	32.5	400	325	3	37	30	2.95	36.6	29.8	98.4	99.1	99.5
5	32.5	400	325	3	37	30	2.95	36.6	29.9	98.4	99.3	99.7
6	32.5	400	325	3	37	30	2.96	36.7	29.9	98.5	99.5	99.8

Table 10: Statistical validation for synthetic mixture

Drug	Mean* %	Standard Deviation*	Co–efficient of Variation* (% R.S.D)	Standard Error*
DEX	98.47	0.018	0.018	0.007
IBU	99.09	0.357	0.36	0.145
PCM	99.43	0.325	0.326	0.132

Table 11: Statistical validation for recovery studies

Level of % Recovery	% Mean Recovery*			Standard Deviation*			Co-efficient of Variation* (% R.S.D.)			Standard Error*		
	DEX	IBU	PCM	DEX	IBU	PCM	DEX	IBU	PCM	DEX	IBU	PCM
80	98.52	99.95	100.22	0.01	0.19	0.17	0.01	0.19	0.169	0.005	0.109	0.098
100	98.55	100.52	100.74	0.015	0.19	0.175	0.015	0.192	0.173	0.008	0.109	0.101
120	98.59	101.09	101.26	0.01	0.195	0.175	0.01	0.197	0.172	0.005	0.112	0.101

Table 12: Statistical validation for intra-day precision

Drug	Mean* (%)	Standard Deviation*	Co–efficient of Variation* (%R.S.D.)	Standard Error*
DEX	99.88	1.145	1.146	0.467
IBU	100.75	0.781	0.775	0.319
PCM	100.32	1.106	1.102	0.451

Table 13: LOD and LOQ Data for Paracetamol, Ibuprofen and Dextromethorphan

Drug	LOD (µg/mL)			LOQ (µg/mL)		
	226 nm	247 nm	279 nm	226 nm	247 nm	279 nm
Paracetamol	1.296	2.482	2.31	3.928	7.521	7
Dextromethorphan	0.979	-	0.994	2.968	-	3.012
Ibuprofen	1.534	-	-	4.65	-	-

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