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Development and Validation of Stability-Indicating HPLC Method for Simultaneous Determination of Related Substances of Acetaminophen and Diphenhydramine Hydrochloride in Rapid release Gel capsules

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ABSTRACT

A simple, precise, accurate, simultaneous and stability-indicating HPLC method developed with an effective resolution of active pharmaceutical ingredients. The present method effectively separates all the related substances of Diphenhydramine hydrochloride and acetaminophen along with impurities. Chromatographic separation has been obtained on Inertsil C18 (250 X 4.6mm, 5µ) column using a gradient elution with a mixture of phosphate buffer pH3.0 and acetonitrile. At 220 nm compounds will be eluted and monitored. Diphenhydramine hydrochloride and acetaminophen were subjected to the stress conditions of acid, base, peroxide, thermal, photolytic, humidity and water degradation. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating ability of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines and validation acceptance criteria were met in all cases. The current method has proven good linearity and accuracy over the range of all known impurities from LOQ to 150% of the target concentration. The degree of reproducibility, as results obtained by deliberate changes in the method parameter and variety of condition has proven that the method is robust and rugged.

Keywords: HPLC, Stability indicating, Acetaminophen, Diphenhydramine Hydrochloride, Related substances.

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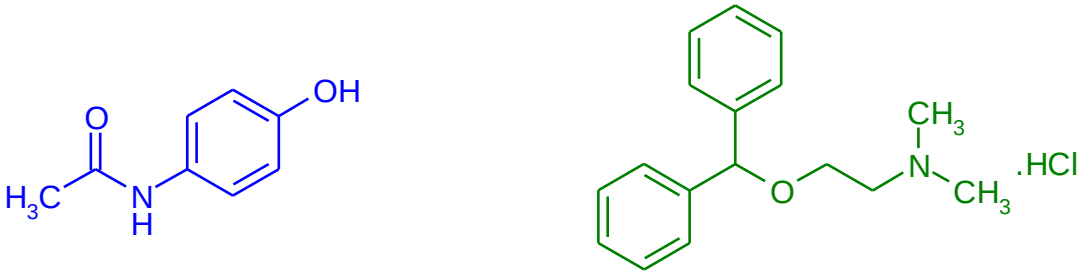
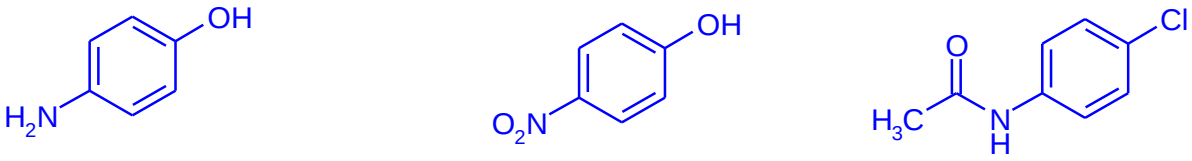
INTRODUCTION

Paracetamol or Acetaminophen[1, 2] (APAP) is used as 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 [3] (Figure.1a) . The main mechanism proposed is the inhibition of cyclooxygenase (COX) and it is highly selective for COX-2. It is having Analgesic and antipyretic properties like NSAIDS (4). Diphenhydramine hydrochloride is 2-(Diphenylmethoxy)-N, N-dimethylethanaminehydrochloride [1, 2] (Figure.1b). It is a white powder and it is having a melting point of 168 °C - 172 °C. It is very soluble in water and freely soluble in alcohol. It has anti-allergic, anti-emetic and sedative properties [5] and when topically applied, Diphenhydramine hydrochloride (diphen) has excellent anesthetic and antipruritic effects. Diphenhydramine hydrochloride has also been shown to be an effective injectable drug for local anesthesia [6.] It is also used in psychiatric medicine to treat phenothiazine drug-induced abnormal muscle movement [7].



(a) (b)

Figure 1: (a) Acetaminophen and (b) Diphenhydramine hydrochloride



4-Aminophenol (Impurity K) 4-Nitrophenol (Impurity J) Chloroacetanilide (Impurity F)

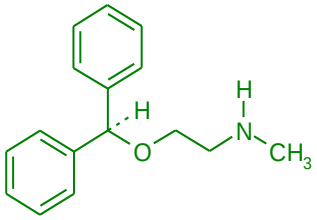
Figure 2: Chemical structures of Acetaminophen impurities

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and finalized formulations is one of the most challenging tasks for pharmaceutical analytical chemists under industrial environment [8]. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As

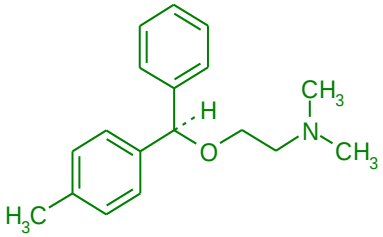
per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product [9].

There are plenty of validated simultaneous analytical methods available for combinational dosage form of acetaminophen and diphenhydramine assay method. But there is no literature available for simultaneous estimation of related substance in combinational dosage form. Spectrophotometric and HPTLC densitometric method for determination of Paracetamol and Diphenhydramine hydrochloride in presence of paracetamol degradation product[10], Paracetamol and Diphenhydramine hydrochloride powder determination by NIR spectroscopy[11], Liquid chromatographic determination of Pseudoephedrine hydrochloride, Acetaminophen, and Dextromethorphen hydrobromide and Diphenhydramine hydrochloride in a compound formulation [12], Micellar liquid chromatographic determination of antihistaminic drugs[13], Simultaneous spectrophotometric determination of paracetamol and diphenhydramine hydrochloride in presence of *p*-aminophenol and *N*-Oxide degradant [14], HPLC Separation of Acetaminophen and its impurities using a mixed-mode reversed-phase/cat ion exchange stationary phase [15], HPLC method for the determination of trace impurities in Acetaminophen drug substance [16], RP-HPLC method for simultaneous determination of Caffeine, Paracetamol and *p*-aminophenol[17], RP-HPLC method for determination of degradants and toxic impurity products of acetylsalicylic acid and paracetamol[18], HPLC separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds with cyanopropyl stationary phase[19] and TLC–Densitometric determination of toxic impurities of Paracetamol and Chlorzoxazone [20] methods were reported.

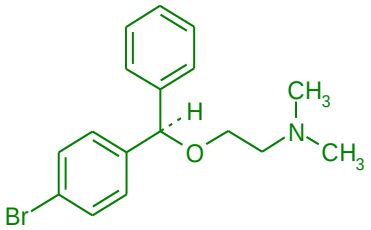
There are many studies available but the reported methods cannot be used for determination of all process-related compounds and degradation products. However, there is no literature available for simultaneous stability indicating method for the combined formulation. So the current work presents simultaneous stability indicating related substance validation using HPLC method. Validation of the method was performed according to the requirements of ICH guidelines for related substance determination, which includes accuracy, precision, selectivity, robustness, linearity and range. Degradation study was performed for active pharmaceutical ingredient, placebo and drug products in acid, base, peroxide, thermal, humidity, and water including photo stability.



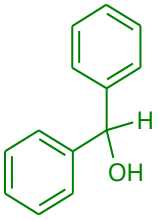
2-(Diphenylmethoxy)-N-methylethanamine (Impurity A)



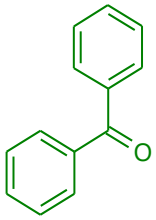
2-[(RS)-(4-methylphenyl) phenylmethoxy]-N, N-dimethylethanamine (Impurity B)



2-[(RS)-(4-bromophenyl)phenylmethoxy]-N,N-dimethylethanamine (Impurity C)



Diphenylmethanol (Benzhydrol) (Impurity D)



Diphenylmethanone (Benzophenone) (Impurity E)

Figure 3: Chemical structures of Diphenhydramine hydrochloride impurities

MATERIALS AND METHOD

Chemicals/Standards/Impurities

Acetonitrile HPLC grade was obtained from Merck. Orthophosphoric acid solution (88%), Potassium di hydrogen phosphate, triethyl amine were from Merck. Acetaminophen and Diphenhydramine Hydrochloride of pharmaceutical grade were purchased from Granules India Limited (Hyderabad, India), were certified to contain 99.87% and 98.82% respectively. Impurities include such as Acetaminophen IMP-K (Impurity K), Acetaminophen IMP-J (Impurity J),

Acetaminophen IMP-F(Impurity F), Diphenhydramine HCl IMP-A(Impurity A), Diphenhydramine HCl IMP-B(Impurity B), Diphenhydramine HCl IMP-C(Impurity C), Diphenhydramine HCl IMP-D(Impurity D) and Diphenhydramine HCl IMP-E(Impurity E). All Impurities were procured from Sigma-Aldrich.

Instrumentation

Analysis was performed on a HPLC (Waters system, Alliance 2695) composed of a quaternary pump, an automatic injector, column thermostat, and temperature-controlled sample trays, an on-line degasser and having a PDA-detector. The control of HPLC system and data processing was done by using class VP software. Ultra-pure water was produced using an ULTRA CLEAR system. The pH of the buffer solutions was measured using a pH/mV-meter Polmon (LP139S), provided with a combined pH electrode. Degradation experiments in acid, alkaline and neutral conditions were performed using a water bath (model EX-35, Neslab Thermo).

Chromatographic Conditions

Chromatographic Separation was achieved on Inertsil C18 (250 X 4.6mm, 5µ) column operated at 35^{o}C with gradient elution at 1ml/min and detection was monitored at wavelength of 220nm. Mobile phase A contains 0.03M phosphate buffer and pH was adjusted to 3±0.02 with phosphoric acid. Mobile phase B contains acetonitrile. The mobile phase was filtered through 0.45 µm membrane filter and degassed ultrasonically before use. Gradient programme was set as time (min)/ mobile phase A: B:: 15/70:30, 25/50:50, 35/50:50, 40/35:65, 50/35:65, 55/70:30, 60/70:30. Mixture of buffer and acetonitrile in the ratio of 30:70 was used as diluents .Injection volume was 10µl.

Preparation of Standard Solutions of Acetaminophen and Diphenhydramine Hydrochloride

Stock standard solutions were prepared by dissolving each of drugs in 0.03M phosphoric Acid: Acetonitrile (30:70) as diluent to achieve a concentration of 10µg/ml.

Preparation of Standard Solutions of Impurities

Standard stock solutions of 250µg/ml were prepared by dissolving 25mg of impurities K,J,F in methanol, in 100ml volumetric flask and volume was made up to mark with water. Standard stock solution of diphenhydramine impurity A was prepared at a concentration of 125µg/ml and impurities B, C, D, E were prepared at concentration of 150µg/ml using same solvent mixture.

Preparation of Analytical Samples

Ten capsules of Sominex (500mg Acetaminophen and 25mg Diphenhydramine Hcl) were weighed the average weight was calculated. An amount of powdered mass equivalent to 250mg of Acetaminophen and 12.5mg of Diphenhydramine Hcl was weighed and transferred into a

250mL volumetric flask. The drugs from powder was extracted and completed to volume with diluent. To ensure complete extraction of drugs it should be sonicated for 10min.Appropriate aliquots from sample stock were suitably diluted with mobile phase to obtain solutions containing 20,000µg/ml of Acetaminophen and 1000 µg/mL of Diphenhydramine hydrochloride. Finally solution was filtered through 0.45µm PVDF filter and degassed.

RESULTS AND DISCUSSION

Method Development

In case of HPLC, various columns are available, but as the main aim of the method was to resolve the compound from degraded products and impurities if any, Inertsil C-18 column (5µm, 250 mm, and 4.6 mm id.) was preferred over the other columns. As per the value of pk_a and solubility of compound, various compositions of mobile phase were tried. Acetaminophen, diphenhydramine hydrochloride and degraded products could not be separated without pH adjustment of the mobile phase. Various pH ranges (2 to 4) for mobile phase were tried, and the best resolution was obtained with pH 3. The chromatographic conditions were optimized with a view to develop a stability indicating assay method, which can separate the drug from its degraded products with good resolution. Mobile phase consisting of acetonitrile and pH 3 phosphate buffer, at a flow rate of 1 mL/min, was found to be satisfactory to obtain well-resolved peaks with better reproducibility and repeatability.

METHOD VALIDATION

After method development, validation of the current test method for Acetaminophen and diphenhydramine hydrochloride gel caps was performed in accordance with United States Pharmacopeia requirements/ICH guidelines for related substance method the parameter includes precision, accuracy, linearity, LOD and LOQ, precision and accuracy at LOQ level, specificity includes blank, placebo, known impurity interference and interference of degradants by degradation study. Robustness and ruggedness was also performed [21, 22.]

System suitability

10µL of standard solution was injected six times it to HPLC system and chromatograms were recorded. % RSD of areas of Acetaminophen, diphenhydramine hydrochloride and their impurities were within the limit of 2.0% and resolution between Acetaminophen and Diphenhydramine hydrochloride was not less than 10.0 for the entire activity. The results are summarized in Table 1(Figure. 4).

Table 1: Results of System Suitability

S. No	Acetaminophen peak	Diphenhydramine Hcl peak
Tailing Factor	1.1	1.0
%RSD of area's	0.27	0.66
Theoretical plates	3291.9	9533.5
Resolution	21.4	

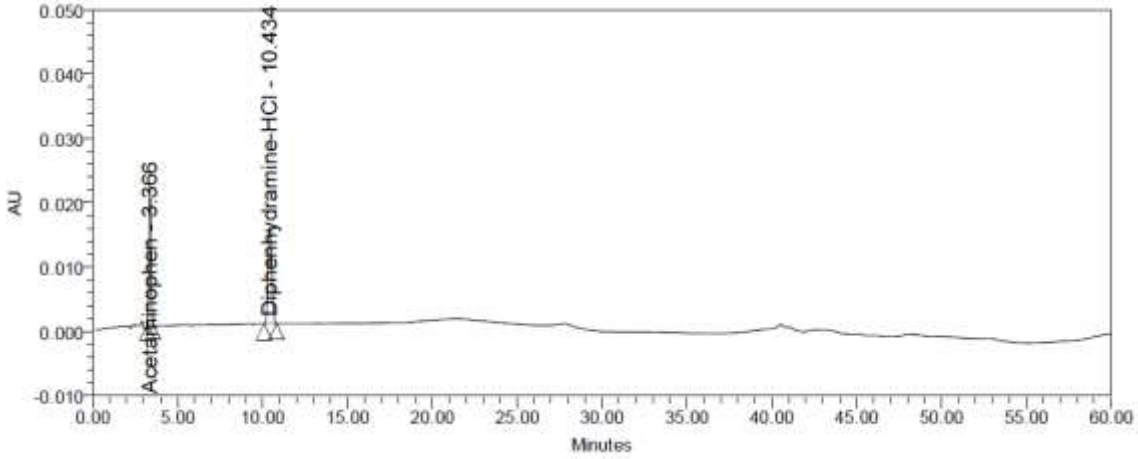


Figure 4: Chromatogram of System Suitability

Precision

To evaluate precision 0.50% of impurity A, 0.30% of impurities B, C, D, E and 0.05% of impurities K, J, F blend has been spiked in the sample preparation and chromatograms were recorded. %RSD was calculated for each individual impurity. The percentage impurity of six replicate sample preparations of impurities A, B, C, D, E and impurities K, J, and F was found to be 0.478, 0.334, 0.310, 0.329, 0.259 and 0.044,0.042 and 0.049 respectively and % RSD was found to be 0.37,1.04,0.85, 0.39, 0.88 and 1.13, 0.41 and 1.81 respectively. The results summarized in Table 2 (Figure 5,6,7).

Table 2: Precision

S.No.	% Impurity							
	IMP-K	IMP-F	IMP-J	IMPA	IMP-B	IMP-C	IMP-D	IMP-E
1	0.044	0.043	0.050	0.481	0.333	0.312	0.331	0.258
2	0.044	0.042	0.050	0.479	0.337	0.313	0.330	0.258
3	0.044	0.042	0.048	0.477	0.330	0.309	0.327	0.257
4	0.044	0.042	0.048	0.476	0.336	0.313	0.329	0.255
5	0.045	0.043	0.050	0.478	0.339	0.307	0.329	0.262
6	0.044	0.042	0.049	0.480	0.332	0.309	0.328	0.261
Avg	0.044	0.042	0.049	0.478	0.334	0.310	0.329	0.259
Std.Dev.	0.0005	0.0001	0.0009	0.0018	0.0035	0.0027	0.0013	0.0023
%RSD	1.13	0.41	1.81	0.37	1.04	0.85	0.39	0.88

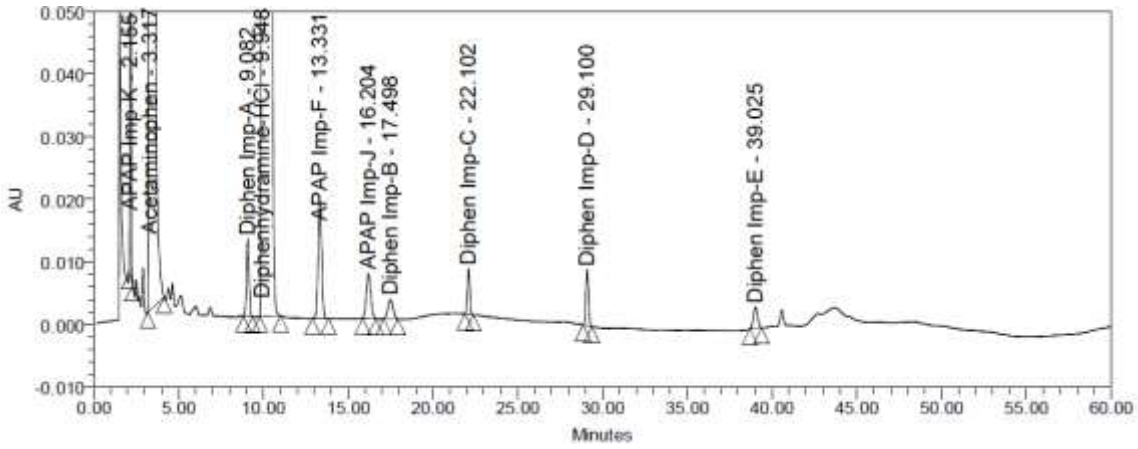


Figure 5: Chromatogram of Test spiked

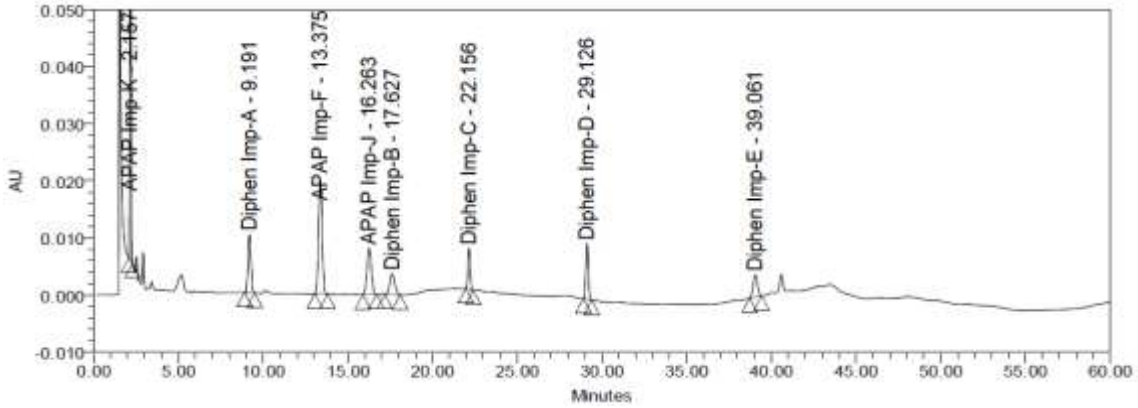


Figure 6: Chromatogram of Placebo spiked

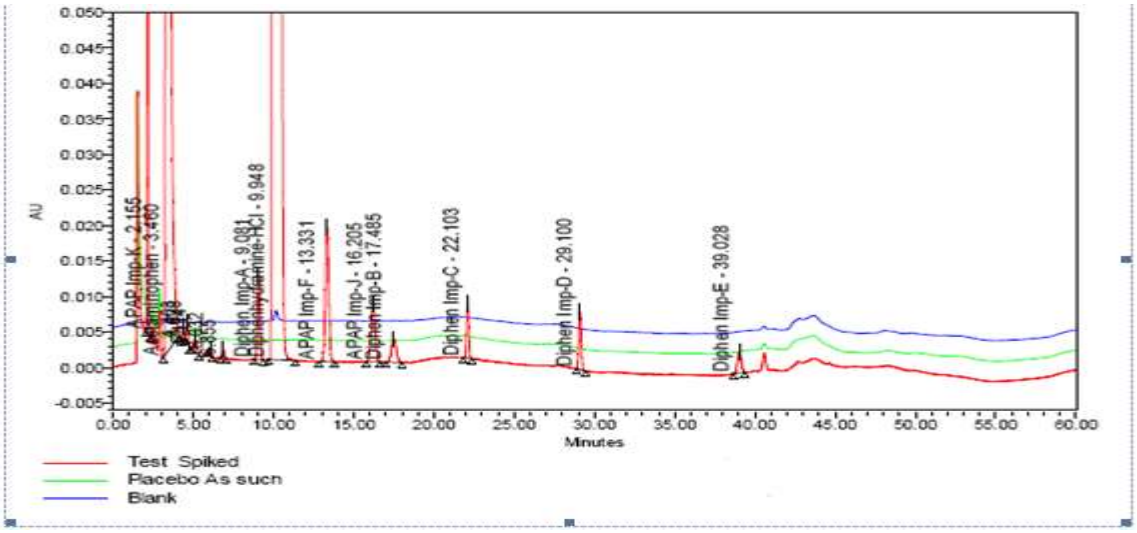


Figure 7: Overlay graph

Intermediate precision

To evaluate intermediate precision, precision experiment was done by different analysts, days, HPLC systems and columns and chromatograms were recorded. %RSD was calculated for each individual impurity. % RSD's of six replicate sample preparations for impurities K ,J, F and A, B,

C, D, E were found to be 2.54, 2.41, 3.34, 5.67, 6.72, 3.89, 3.67 and 3.36 respectively. The results are summarized in Table 3.

Table 3: Intermediate Precision

	S.No.	Spiked Sample-Unspiked Sample (%)							
		IMP-K	IMP-F	IMP-J	IMP-A	IMP-B	IMP-C	IMP-D	IMP-E
PRECISION STUDY	1	0.044	0.043	0.050	0.481	0.333	0.312	0.331	0.258
	2	0.044	0.042	0.050	0.479	0.337	0.313	0.330	0.258
	3	0.044	0.042	0.048	0.477	0.330	0.309	0.327	0.257
	4	0.044	0.042	0.048	0.476	0.336	0.313	0.329	0.255
	5	0.045	0.043	0.050	0.478	0.339	0.307	0.329	0.262
	6	0.044	0.042	0.049	0.480	0.332	0.309	0.328	0.261
RUGGEDNESS STUDY	1	0.043	0.045	0.048	0.520	0.359	0.338	0.354	0.281
	2	0.042	0.045	0.046	0.520	0.362	0.337	0.351	0.272
	3	0.042	0.045	0.048	0.524	0.318	0.340	0.354	0.278
	4	0.042	0.043	0.046	0.440	0.304	0.315	0.318	0.257
	5	0.042	0.043	0.046	0.441	0.298	0.316	0.322	0.260
	6	0.042	0.044	0.047	0.482	0.291	0.325	0.334	0.271
Average		0.042	0.044	0.047	0.488	0.322	0.329	0.339	0.270
Stdev		0.0003	0.0007	0.0010	0.039	0.0311	0.0115	0.0165	0.0097
%RSD		0.83	1.69	2.07	8.18	9.66	3.51	4.87	3.59
Overall Avg		0.043	0.043	0.048	0.483	0.328	0.319	0.334	0.264
Overall Stdev		0.0011	0.0010	0.0016	0.0274	0.0221	0.0124	0.0123	0.0089
Overall %RSD		2.54	2.41	3.34	5.67	6.72	3.89	3.67	3.36

Accuracy

Accuracy of the method was studied for three levels from 50% to 150% by spiking 0.25 %, 0.15% for 50% level from the target concentration for Diphenhydramine impurities A and B,C,D,E followed by 0.025% for Acetaminophen impurities K, J, F and 0.50%, 0.30%, 0.05% for 100% and 0.75% , 0.45% and 0.075% respectively for 150% level. All impurities have been spiked in sample preparation and Chromatogram's of spiked sample preparation has been compared with unspiked sample preparation. Six replicate preparations have been made for each level. Recovery was found between 94.6% to 109.5 % and RSD was found between 0.19% to 2.96%. Results are summarized in Table 4.

Table 4: Accuracy

% Impurity								
Acetaminophen				Diphenhydramine Hydrochloride				
Levels	K	J	F	A	B	C	D	E
50%	96.5	94.4	100.2	103.3	98.4	101	101.7	98.6
	94.8	96.7	102.2	105.0	101.1	102.8	103.5	98.2
	92.9	96.5	100.3	104.3	100.8	103.2	102.9	98.5
%RSD	1.87	1.36	1.15	0.79	1.48	1.14	0.90	0.24

100%	94.6	98.8	100.0	104.3	100.2	101.3	100.8	97.7
	94.8	98.8	100.8	104.8	100.2	101.5	101.5	99.1
	94.3	99.8	101.0	104.8	100.5	102	101.5	98.7
%RSD	0.25	0.57	0.52	0.30	0.19	0.33	0.36	0.72
150%	104.1	104.6	108.0	113.1	106	109.2	108	103.4
	99.7	100.4	102.7	106.9	101.9	103.6	103.4	97.4
	100.7	102.2	104.4	108.5	103.3	105.1	104.7	99.7
%RSD	2.29	2.07	2.561	2.96	2.04	2.93	2.26	3.02

Limits of detection and quantification

Limits of detection (LOD) were calculated as the concentration of the analyte which gave a peak 3 times higher than the baseline noise. The LODs found were:0.469 µg/mL for Acetaminophen,0.047µg/mL for Imp-K, 0.085µg/mL for Imp-F, 0.140 µg/mL for Imp-J, 0.043µg/mL for Diphenhydramine Hydrochloride, 0.073µg/mL for Imp-A, 0.027µg/mL for Imp-B, 0.084 µg/mL for Imp-C, 0.061µg/mL for Imp-D and 0.096µg/mL for Imp-E respectively. Limit of quantification (LOQ) was calculated as the concentration of the analyte which gave a peak 10 times higher than the baseline noise. The LOQs determined were: 1.421µg/mL for Acetaminophen,0.144µg/mL for Imp-K, 0.259µg/mL for Imp-F, 0.425µg/mL for Imp-J, 0.130µg/mL for Diphenhydramine Hydrochloride, 0.222µg/mL for Imp-A, 0.082µg/mL for Imp-B, 0.254µg/mLfor Imp-C, 0.186µg/mL for Imp-D and 0.293µg/mL for Imp-E respectively. Results are summarized in Table 5.

Table 5: LOD & LOQ

Impurities	**µg/mL**	
	LOD	**LOQ**
Impurity K	0.0476	0.1444
Impurity F	0.0856	0.2594
Impurity J	0.1403	0.4251
Acetaminophen	0.469	1.4217
Diphenhydramine Hcl	0.0431	0.1307
Impurity A	0.0733	0.2221
Impurity B	0.0273	0.0828
Impurity C	0.0840	0.2545
Impurity D	0.0616	0.1865
Impurity E	0.0967	0.2930

Linearity

To evaluate linearity of the method, six levels calibration curve made includes LOQ level. Signal to noise ratio was observed about 10 for the concentration between 0.05 to 10µg/mL for Diphenhydramine hydrochloride, Acetaminophen and its impurities. Hence linearity was established LOQ to 30 µg/mL for Diphenhydramine hydrochloride, Acetaminophen and its

impurities. The correlation coefficient (R) of Impurities K, F, J, Acetaminophen and impurities A, B, C, D, E and Diphenhydramine hydrochloride was found 0.9999,0.9999,0.9998,0.9998 and 0.9995,0.9990,0.9995,0.9996,0.9988 respectively. The results are summarized in table 6 and overall linearity graph for Diphenhydramine hydrochloride, Acetaminophen and its impurities was shown in Figure 15 & 16.

Table 6: Linearity

Impurities	Correlation Coefficient (R)	%RSD of Lower level	%RSD of Higher level
Impurity K	0.9999	1.55	0.46
Impurity F	0.9999	2.48	0.59
Impurity J	0.9998	5.02	0.89
Acetaminophen	0.9998	0.89	0.36
Diphenhydramine HCl	0.9990	6.45	1.68
Impurity A	0.9995	2.54	0.42
Impurity B	0.9990	9.21	2.27
Impurity C	0.9995	2.38	0.98
Impurity D	0.9996	5.20	0.60
Impurity E	0.9988	6.10	0.63

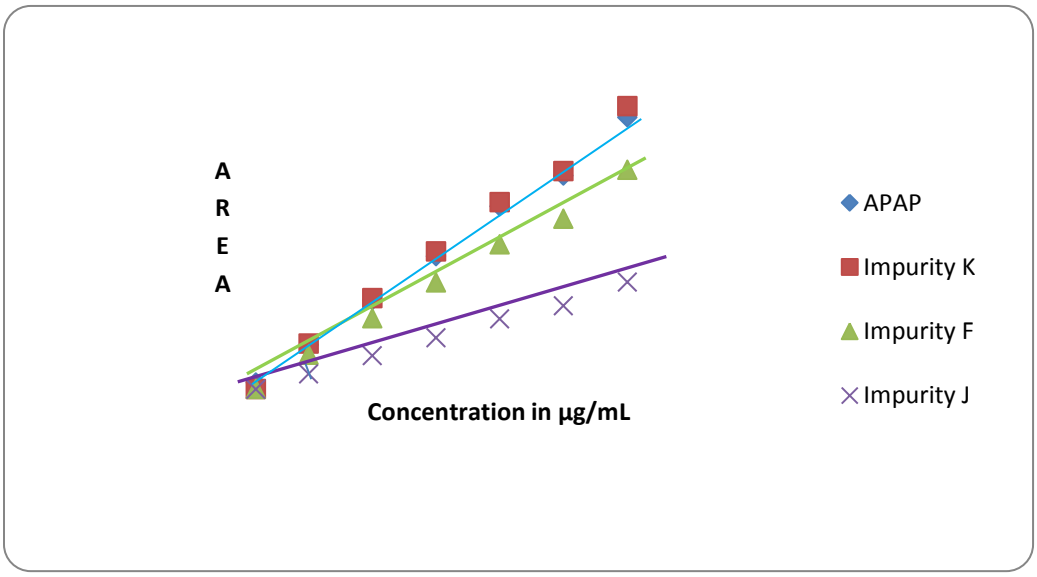


Figure 15: Linearity graph of Acetaminophen and its impurities

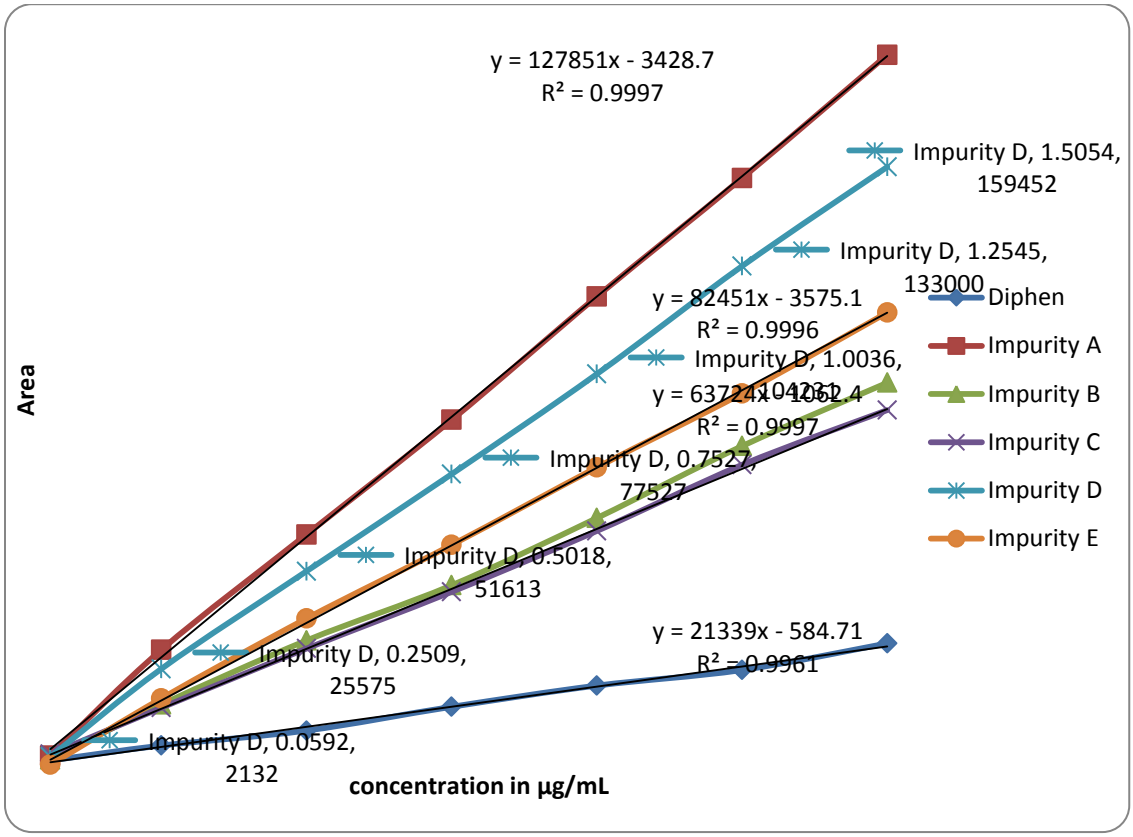


Figure 16: Linearity graph of Diphenhydramine and its impurities

Robustness

Robustness of the current method was investigated by analysing the sample solution and established system suitability with the deliberate variation of mobile phase P^H at 0.2 units, wavelength at 5nm and column temperature at 5^oC from the original value. The overall % RSD from 6 sample preparations of precision study (method precision)and sample preparations from robustness together shall not be more than 10.0% The results are summarized in Table 7

Table 7: Robustness

% RSD							
Temperature				**Wavelength**		**pH**	
S.No	Impurities	30^0C	40^0 C	217	227	2.8	3.2
1	K	2.68	4.94	1.64	5.49	2.01	3.34
2	F	2.27	2.39	6.60	4.25	5.10	0.53
3	J	3.07	3.42	3.51	1.72	2.17	2.02
4	A	8.23	7.85	6.43	5.88	7.94	4.51
5	B	0.95	2.01	7.31	2.72	0.96	1.03
6	C	2.06	2.25	3.03	1.75	2.21	4.19
7	D	0.47	0.38	2.28	5.52	0.48	1.75
8	E	9.27	5.15	8.53	6.29	2.37	8.48

Stability studies

Forced degradation studies of both the drugs were carried out under conditions of acid/base hydrolysis, oxidation and thermal hydrolysis, photolytic and humidity hydrolysis. The drugs were subjected to acid hydrolysis by using 1 M hydrochloric acid and base hydrolysis by using 1 N sodium hydroxide solution; oxidation by using 30% hydrogen peroxide solution and thermal hydrolysis at 50 ^{0}C: photolytic at 360nm for 7 days and humidity at 75% RH for 7 days. The stress conditions varied both in terms of temperature and time to achieve the appropriate degradation . After the degradation treatments were completed, the stress content solutions were allowed to room temperature and diluted with mobile phase up to the mark. Filter the solutions with 0.45 microns filters and injected to column under proposed conditions. Peak purity of analyte peaks should be less than purity threshold. Results are tabulated in Table 8, 9(Figure. 8, 9, 10, 11, 12, 13, 14).

Table 8: Stability Studies of Acetaminophen

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	---	12.783	25.001
Thermal Stress Sample	0.8	13.271	25.001
Humidity Stress Sample	0.7	12.831	25.001
Photolytic Stress Sample	0.2	13.035	25.001
Water Stress Sample	23.4	12.279	25.001
Acid Degradation (1.0 N Hcl)	26.3	12.259	25.001
Alkali Degradation (1.0 N NaOH)	25.6	12.656	25.001
Peroxide Degradation (30% v/v)	26.6	11.879	25.001

Table 9: Stability Studies of Diphenhydramine Hydrochloride

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	---	5.245	25.005
Thermal Stress Sample	3.1	4.999	25.007
Humidity Stress Sample	Nil	5.429	25.006
Photolytic Stress Sample	3.2	5.333	25.007
Water Stress Sample	6.0	4.574	25.007
Acid Degradation (1.0 N Hcl)	96.1	0.131	25.080
Alkali Degradation (1.0 N NaOH)	Nil	5.594	25.005
Peroxide Degradation (30% v/v)	27.0	4.187	25.007

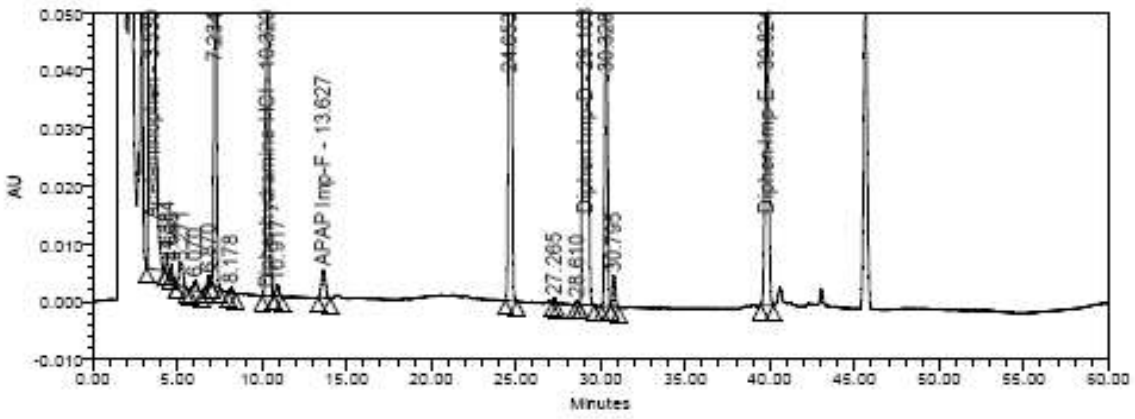


Figure 8: Chromatogram of Acid Hydrolysis

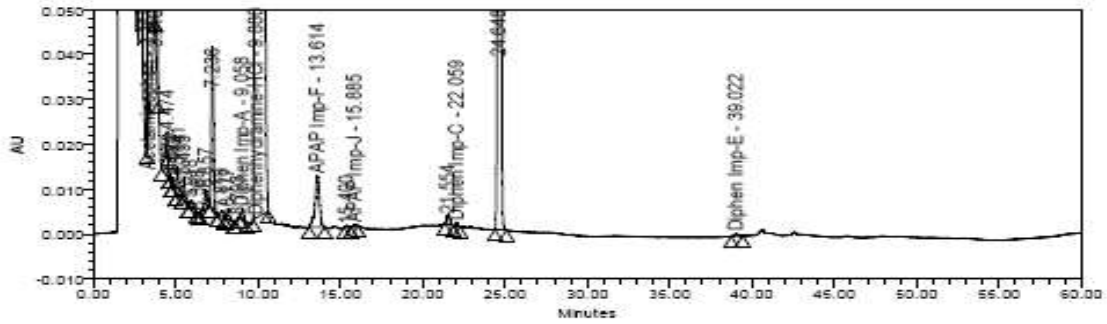


Figure 9: Chromatogram of Alkali Hydrolysis

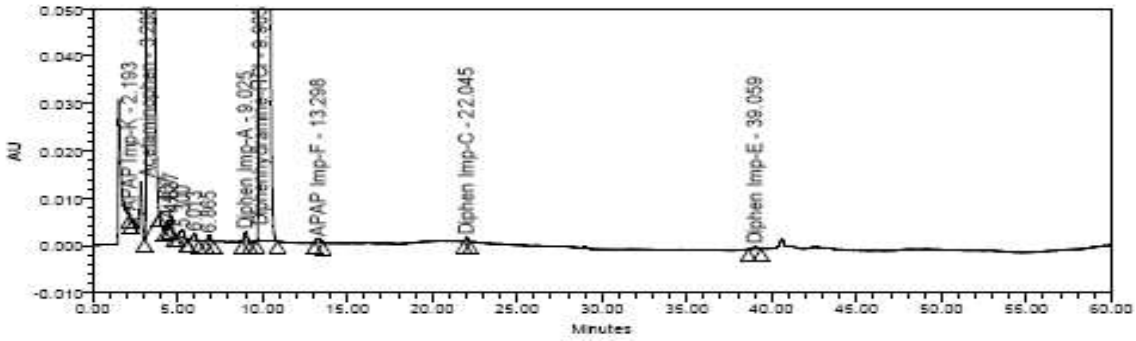


Figure 10: Chromatogram of Photolytic Degradation

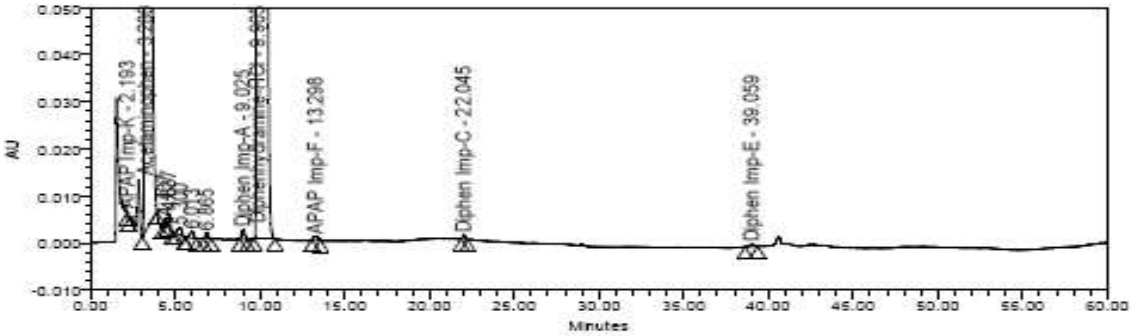


Figure 11: Chromatogram of Humidity Degradation

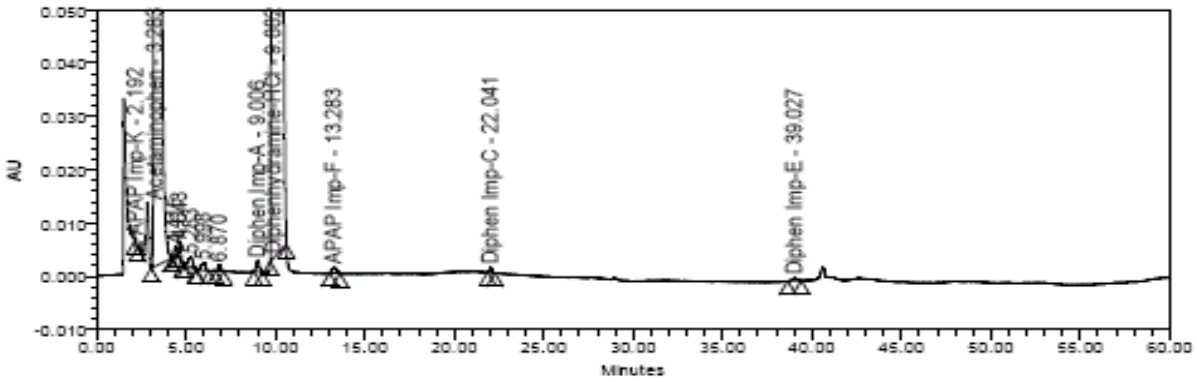


Figure 12: Chromatogram of Thermal Degradation

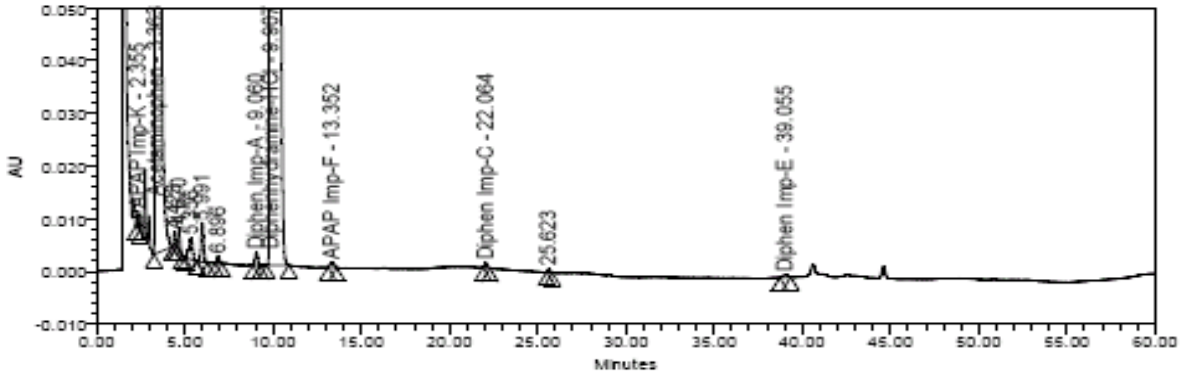


Figure 13: Chromatogram of Water Degradation

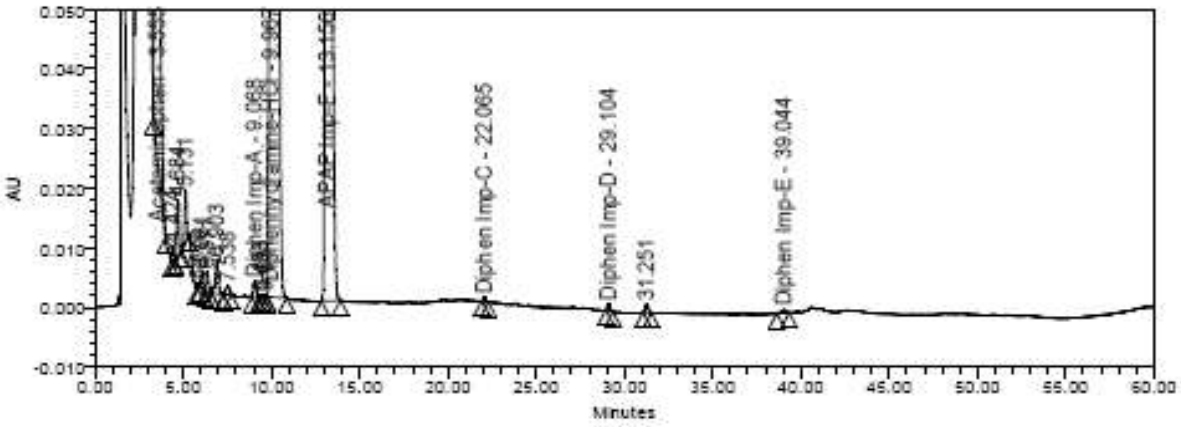


Figure 14: Chromatogram of Peroxide Oxidation

CONCLUSION

A simple, accurate, precise, simultaneous and stability-indicating HPLC method was developed and validated for the routine analysis of related compounds of acetaminophen and diphenhydramine hydrochloride combined formulation. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability-indicating.

REFERENCES

1. The United States Pharmacopoeia 35 and National Formulary 35, American pharmaceutical Association, Washington, DC, 2012.
2. The British Pharmacopoeia, Her Majesty's, The Stationary Office, London, 2007.
3. Moller. PL, Sindet-Pedersen. S, Petersen. CT, Juhi. GI, Diltenschneider. A, Skoglund. LA. Onset of acetaminophen analgesia: comparison of oral and intravenous routes after third molar surgery. Br J Anaesth. 2005; 94: 642-648.
4. Hinz. B, Cheremina. O, Brune. K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. FASEB J. 2008; 22: 383-390.
5. Adolph. O, Georgieff. M, Zeller. K, Fohr. K.J. Diphenhydramine inhibits NMDA-induced currents- New pharmacological aspects of a well-known drug. European Journal of Anesthesiology. 2014; 31: 142.
6. Peter G. Pavlidakey. MD, Erin E. Brodell, Stephen E. Helms, 2009. Diphenhydramine as an Alternative Local Anesthetic Agent. J Clin Aesthet Dermatol. 2009; 2: 37-40.
7. http://www.encyclopedia.com/topic/Diphenhydramine.aspx.
8. Gorog, S, Babjak, M, Balogh, G, Brlik, J, Csehi, A, Dravecz, F, Gasdag, M, Horvath, P, Lauko, A, Varga, K. Drug impurity profiling strategies. Talanta. 1997; 44: 1517-1526.
9. "Impurities in new drug substances Q3A (R2),"Current Step 4 Version, Parent Guidelines on Methodology Dated October 25 2006. International Conference on Harmonization, Geneva, Switzerland.
10. Nourudin W, Ali, Hala E, Zaazaa, Abdelkawy, M, Maimana A, Magdy. Simultaneous Determination of Paracetamol and Diphenhydramine Hydrochloride in Presence of Paracetamol Degradation Product. Pharmaceutical Analytical Acta. 2011; 2: 1-8.
11. Ying Dou, Ying Sun, Yuqiu Ren, Yulin Ren. Artificial neural network for simultaneous determination of two components of compound paracetamol and diphenhydramine Hydrochloride powder on NIR spectroscopy. Analytica Chimica Acta. 2005; 528: 55-61.
12. Meiling Qi, Peng Wang, Li Zhou, Yan Sun. Simultaneous Determination of Four Active Components in a Compound Formulation by Liquid Chromatography. Chromatographia.2003; 58: 183-186.

13. Martinez-Algaba, C, Bermudez-Saldana, J.M, Villanueva-Camanas, R.M, Sagrado, S, Medina Hernandez, M.J. Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography. Journal of Pharmaceutical Biomedical Analysis.2006; 40: 312-321.
14. NouruddinW. Ali, Abdelkawy. M, Nessreen S. Abdelhamid. Simultaneous determination of paracetamol and diphenhydramine hydrochloride mixture in the presence of their degradation products. IOSR Journal of Pharmacy and Biological Sciences.2013; 6: 44-52.
15. Octavian Calinescu, Irinel A. Badea, Luminita Vladescu, Viorica Meltzer, Elena Pincu. HPLC Separation of Acetaminophen and its Impurities Using A Mixed-mode Reversed-Phase/Cation Exchange Stationary Phase. Journal of Chromatographic Science.2011; 50: 335–342.
16. Marika Kamberi, Christopher M.Riley, Xiaoyan Ma, Chen-wen C Huang . A validated sensitive HPLC method for the determination of trace impurities in acetaminophen drug substance. Journal of Pharmaceutical and Biomedical Analysis.2004; 34: 123-128.
17. Milkica Crevar, Branka Ivkovic, Sote Vladimirov, Vesna Kuntic, Zorica Vujic. Statistical Optimization of Reverse Phase High Performance Liquid Chromatography for the Analysis of Caffeine Paracetamol and its Degradation Product *p*-aminophenol. Acta Chim.Slov.2008; 55: 665-670.
18. Cemal Akay, Ismail Tuncer Degim, Ahmet Sayal, Ahmet Aydin, Yalcin Ozkan, Husamettin Gul. Rapid and Simultaneous Determination of Acetylsalicylic Acid, Paracetamol, and Their Degradation and Toxic Impurity Products by HPLC in Pharmaceutical Dosage Forms. Turk J Med Sci.2008; 38:167-173.
19. Olmo.B, Garcıa .A, Marın. A, Barbas .C. New approaches with two cyano columns to the separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds. Journal of Chromatography B.2005; 817: 159-165.
20. Ali MS, Rafiuddin S, Ghori M, Kahtri AR. Simultaneous determination of paracetamol, chlorzoxazone, and related impurities 4-aminophenol, 4'-chloroacetanilide, and p-chlorophenol in pharmaceutical preparations by high-performance liquid chromatography. Journal of AOAC International. 2007; 90: 82-93.
21. ICH, 2005. International Conference on Harmonization (ICH) Q2 (R1): Validation of Analytical Procedures—Test and Methodology. Geneva, Switzerland, 2005.

22. ICH, 2005. International Conference on Harmonization: Draft Revised Guidance on Q1A (R) Stability Resting of New Drug Substances and Products. Federal Register 65 (2000) 21446.

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