IJPSR (2021), Volume 12, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 January 2021; received in revised form, 22 August 2021; accepted, 12 October 2021; published 01 December 2021

STABILITY INDICATING HPLC METHOD FOR PARACETAMOL, CAFFEINE, PHENYLEPHRINE HCL, CHLORPHENAMINE MALEATE AND ITS IMPURITIES IN FLUCOLD TABLET DOSAGE FORM

Ganpisetti Srinivasa Rao [1, * 2], D. Rama Devi [3], P. Sunil Reddy [1], L. Kalyanaraman [1], Parimalkumar Sureshbhai Patel [1], B. Venkata Subbaiah [1], B. M. Rao [1, 2], Nagaraju Rajana [2] and K. Basavaiah [2]

Dr. Reddy's, Laboratories [1], Bachupally, Hyderabad - 502325, Telangana, India.
Department of Chemistry [2], AU College of Pharmaceutical Sciences [3], Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Tablets dosage form, Method development, Validation, Degradation

Correspondence to Author:
Ganpisetti Srinivasa Rao

Dr. Reddy's, Laboratories, Bachupally, Hyderabad - 502325, Telangana, India.

E-mail: srinivasarg@drreddys.com

ABSTRACT: Paracetamol, caffeine, phenylephrine, and chlorphenamine maleate are available on the market to treat pains, psychoactive disorders, and bronchopulmonary disorders. The objective was to develop a single HPLC method to determine paracetamol, caffeine, phenylephrine HCl, chlorphenamine maleate, and its impurities in flu cold tablets combination dosage form. No analytical method is available to assess the quality of flu cold tablets' combination dosage form; hence the present research was taken. The evaluated method was validated with Inert sustain C18 AQ 250 mm x4.6 mm, 3µm column, flow rate of 0.7 mL/min, 220 nm wavelength, 50 µL injection volume, 35 °C column temperature and gradient program run time 160 min and also used the pH 2.5 and 4.0 phosphate buffer. This single method was developed and validated with precision, accuracy, ruggedness, linearity, robustness, and specificity by following ICH and USP validation of compendial procedures. The degradation study was performed at all stress conditions such as water hydrolysis, acid hydrolysis, base hydrolysis, oxidation, thermal and light exposure, the peak purity of each active pharmaceutical ingredient in combination dosage form less than peak threshold, which indicates that developed and validated HPLC method was stability-indicating.

INTRODUCTION: Paracetamol is also called acetaminophen and APAP. Paracetamol's chemical name is 4- hydroxyl acetanilide. It is used to treat pain and fever and works as centrally and peripherally acting non-opioid analgesic and antipyretic drug [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(12).6424-31

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6424-31

Paracetamol is available in market in different dosage forms such as tablets, capsules, drops, elixirs, suspension and suppositories. The maximum daily dose for adults is 3-4 grams [2].

Caffeine belongs to the central nervous system (CNS) stimulant of methylxanthine class and widely used psychoactive drug [3]. Caffeine has both positive and negative health effects. It can be used to treat premature infant breathing disorders bronchopulmonary dysplasia [4]. Chlorphenamine is the first generation antihistamine drug [5]. The general application is allergic symptoms prevention such as rhinitis and urticarial [6].

Phenylephrine is phenethylamine class medicine and is used as a decongestant [7-8]. It is an agent to dilate the pupil to increase blood pressure and relieve haemorrhoids. Four medicinal products are available in the market in tablets, syrups and injection dosage forms. Individual and combination products are available.

Paracetamol, caffeine, phenylephrine HCl and chlorphenamine maleate tablets (500 mg + 30 mg + 5 mg + 2 mg) are new combination product and available in the market **Fig. 1**. The chemical structure of four active components and their impurities are represented in **Table 1.**

TABLE 1: STRUCTURES AND ITS THERAPEUTIC ACTIVITIES OF SELECTED DRUGS

Name of Compound	Structure	M. wt	Therapeutic activities
Paracetamol		151.17	Medication used to treat pain and fever
Paracetamol Impurity-F		139.11	NA
Paracetamol Impurity-J		169.61	NA
Paracetamol Impurity-K		109.13	NA
Caffeine		194.19	A central nervous system stimulant of the methylxanthine class
Caffeine Impurity-A		180.17	NA
Caffeine Impurity-E		168.20	NA
Phenylephrine HCl		203.67	Medication used as a decongestant
Phenylephrine HCl Impurity-C		201.65	NA
Phenylephrine HCl Impurity-E		255.32	NA
Chlorphenamine Maleate		390.86	Medication used to treat the symptoms of allergic conditions
Chlorphenamine Maleate Impurity-C		376.84	NA
Chlorphenamine Maleate Impurity-E		415.87	NA

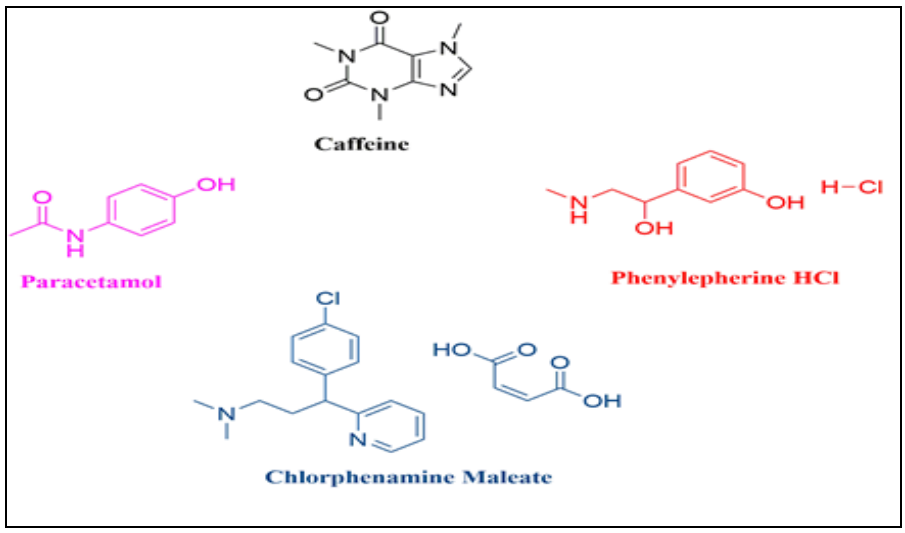

FIG. 1: CHEMICAL STRUCTURE OF THE FOUR ACTIVE COMPONENTS AND THEIR IMPURITIES

Impurities chemical names are Paracetamol imp-J: N-(4-chlorophenyl) acetamide; Paracetamol imp-K: 4-aminophenol; Paracetamol imp-F: 4-nitrophenol; Caffeine imp-A: 1, 3-dimethyl-1H-purine-2, 6 (3H,9H)-dione; Caffeine imp-E:N,1-dimethyl-4-(methylamino) - 1H - imidazole - 5-carboxamide; PE imp-C: 1 - (3 - hydroxyphenyl) – 2 -(methyl-amino) ethanone hydrochloride; PE imp-E: 1-(3-hydroxyphenyl)-2-(methyl (phenethyl) amino) ethanone; CPM imp-C: 3-(4-chlorophenyl)-N-methyl-3-(pyridin-2-yl)propan-1-amine maleate and CPM imp-D: 2-(4-chlorophenyl)-4-(dimethylamino)-2-(pyridin-2-yl) butanenitrile maleate. Literature survey was performed and found multiple methods to determine each component in an individual product and combination with other ingredients. No single method is reported to determine these four active medicines in the same combination product [9-23]. Hence, the main objective of this research work is to develop a single HPLC method to determine the four active drugs and their related impurities.

MATERIALS AND METHOD:

Chemicals Reagents: Analytical reagent grade tetrabutylammonium hydrogen sulphate (TBAHS) purchased from Rankem (Mumbai, India). HPLC grade methanol purchased from Rankem and Millipore Milli Q purification system purchased from Bangalore, India.

HPLC Instrument: Waters Alliance 2695 separations module equipped with gradient elution capability, 2487 UV detector, and an autosampler. Empower work station data handling system.

Chromatographic Conditions: Inert Sustain C18 AQ250x4.6 mm, 3µm equivalent column, flow rate 0.7mL/min, 220 nm wavelength, 50 µL injection volume, column temperature 35 °C were used. Run time 160 min was performed. The gradient program is represented in **Table 1**. Mobile phase A was pH 2.5 potassium di-hydrogen phosphate buffer; mobile phase B was Mix accurately 950 mL of acetonitrile with 50 mL of water and mobile phase CpH 4.0 potassium di-hydrogen phosphate buffer. Diluent was 950 mL of 2.5 pH buffer and 50 ml acetonitrile.

Solutions Preparations: Standard solution:(PE and CPM) 50.0 mg of PE HCl and 64 mg CPM standards into 100 Ml volumetric flask, 60mLof diluent added and sonicated to dissolve and made up to volume with diluent and mixed. 5mL of above solution into 100 ml diluted with diluent. Again diluted 4mL of this solution into 100 ml with diluent.

CPM Impurity C Stock: 3mg of CMP impurity C standard transferred in to 50mLand diluted with diluent.

Resolution Solution: 10mg of CPM in 50mL volumetric flask and add 1.0Ml of CPM impurity C stock and dilute with diluent.

Sample Solution (PE and CPM): 10 tablets dropped into 100Ml volumetric flask, 60mL of diluent added and sonicated for 25min and diluted. The standard solution chromatograms are represented in **Fig. 2.**

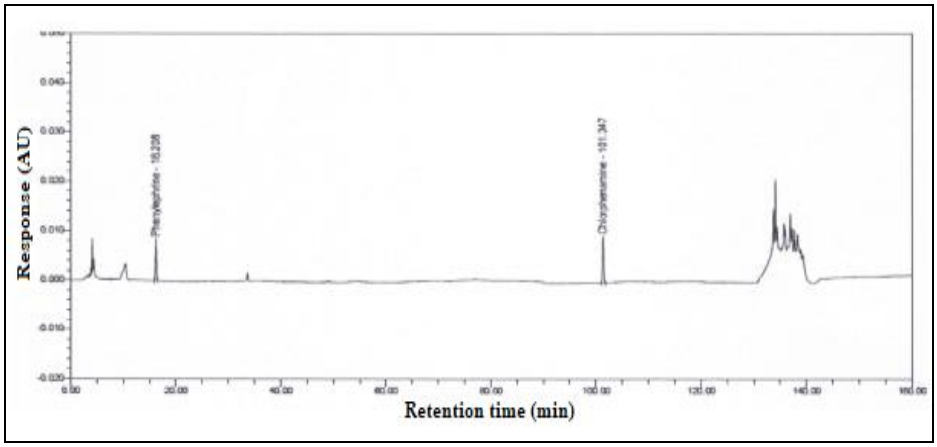

FIG. 2: STANDARD SOLUTION CHROMATOGRAM OF PE: PHENYLEPHRINE, CP: CHLORPHENAMINE

Preparation of Phenylephrine Hydrochloride and Chlorpheniramine Maleate Standard Stock Solution: Weighed accurately and transferred about 125 mg of phenylephrine hydrochloride working standard or reference standard, 50 mg of chlorpheniramine Maleate working standard or reference standard into a 50 mL volumetric flask, add 35 mL diluent, sonicate to dissolve and made up to mark with diluent and mixed well.

Preparation of Standard Stock Solution: Weighed accurately and transferred about 250 mg of paracetamol working standard or reference standard and 75 mg of caffeine working standard or reference standard in 50mL volumetric flask to its pipette 5 mL of phenylephrine hydrochloride and chlorpheniramine maleate standard stock solution and 35mL diluent, sonicate to dissolve and made up to mark with diluent and mixed well.

Preparation of Standard-1 Solution: Concentration is about phenylepherine hydrochloride (1.25 µg/mL equivalent to 0.5% of 250 µg/mL), chlorphenaramine maleate (0.5 µg/mL equivalent to 0.5% of 100 µg/mL), caffeine (7.5 µg/mL equivalent to 0.5% of 1500 µg/mL) and paracetamol (25 µg/mL equivalent to 0.1% of 25000 µg/mL). Pipette 5 mL of standard stock solution in to a 100 mL volumetric flask and made up to mark with diluent and mixed well. Pipette 5 mL of above solution in to a 50 mL volumetric flask and made up to mark with diluent and mixed well.

Preparation of Standard-2 Solution: Pipette 5 mL of standard-1 solution into a 50 mL volumetric flask and made up to mark with diluent and mixed well.

Preparation of test Solution: 497.44 mg of paracetamol, 36.79 mg of caffeine, 6.49 mg of phenylephrine HCl, 2.189 mg of cholr-phenaramine maleate, and 113.36 mg of placebo were transferred in 20 mL volumetric flask to it add 15 mL of diluent and sonicated for 20 min and diluted up to the mark with diluent and centrifuged the solution at 4000 rpm for 10 minutes and injected into HPLC.

RESULTS AND DISCUSSION:

Method Development and Optimization: Literature published reports were understood and evaluated the optimized methods for the determination and impurity profiling, but no method was reported to determine the four combination products. Eventually, we started the HPLC method development based on the chemical and physical properties of the analytes.

In development trial-1, the chromatographic conditions were the mobile phase-A: Buffer (1.36g KH2PO4 in 1-liter water pH 2.50 with OPA). Mobile phase-B: methanol. Diluent: water, methanol 50:50 v/v. Inertsil C18 250 x 4.6mm,5µ column, 1.0 mL/min, 215 and 225nm, 35°C, 20µL and runtime 90min. Gradient program: M.P-A at 0 min 95%, 10 min 95%, 35 min 82%, 72 min 52%, 77 min 35%, 78 min 95% and 90 min 95%. Paracetamol impurity K was not retained; phenylephrine and impurity C were co-eluted and had baseline noise. In development trial-2, the chromatographic conditions were the mobile phase-A: buffer (1.36g KH2PO4 in 1-liter water pH 7.0 with KOH). Other conditions were as per trial-1. All impurities were separated, but the Caffeine impurity peak shape was poor. In development

trial-3, the chromatographic conditions were the mobile phase-A: buffer (1.36g KH2PO4 in 1-liter water pH 2.50 with OPA). Mobile phase-B: 1 mL OPA in 500 mL methanol. Diluent: water, methanol 50:50 v/v. Inertsil C18 250 x 4.6mm,5µ column, 1.0mL/min, 225nm, 35°C, 20µL and runtime 90min. Gradient program: M.P-A at 0 min 95%, 10 min 95%, 35 min 78%, 72 min 45%, 77 min 25%, 82 min 25%, 85 min 95% and 95 min 95% . Chlorphenamine A and B were co-eluted. In development trial-4, the chromate-graphic conditions were the mobile phase-A: buffer (1.36g KH2PO4 in 1-liter water pH 2.50 with OPA). Mobile phase-B: methanol.

Diluent: water, methanol 50:50 v/v. Inertsil C18 250*4.6mm, 5µ, 1.0mL/min, 225nm, 35°C, 20µL, and runtime 90min. Gradient program: M.P-A at 0 min 95%, 10 min 95%, 35 min 82%, 72 min 52%, 77 min 35%, 78 min 95% and 90 min 95%. Paracetamol impurity K was not retained, and phenylephrine and impurity C were co-eluted and had baseline noise. In development trial-5, the chromatogram conditions were the mobile phase-A: buffer (1.36g KH2PO4 in 1-liter water pH 7.0 with KOH). Mobile phase-B: methanol.

Diluent: water, methanol 50:50 v/v. Inertsil ODS 3V 250 x 4.6mm,5µ column, 1.0 mL/min, 225nm, 40°C, 20µL and runtime 90min. Gradient program: M.P-A at 0 min 100%, 12 min 100%, 70 min 76%, 75 min 70%, 80 min 35%, 86 min 35%, 88 min 100% and 100 min 100%. The four product peaks were eluted with good peak shape, but gradient noise was observed. In development trial-6, the chromatographic conditions were buffer (1.36g KH2PO4 in 1 liter water pH 7.0 with KOH).Mobile phase-A: 950 buffer, 50 methanol.

Mobile phase-B: ACN, Water, OPA 950: 50: 2. Diluent: water, methanol 50:50 v/v. Inertsil ODS 3V 250 x 4.6mm,5µ column, 1.0mL/min, 225nm, 40°C, 40µL and runtime 90min. Gradient program: M.P-A at 0 min 100%, 10 min 100%, 75 min 76%, 85 min 70%, 88 min 70%, 92 min 10%, 100 min 10%, 105 min 100 % and 120 min 100%. Injection volume was evaluated and found that 50µL is suitable for establishing the LOD, LOQ parameters, and accurate quantification in routine analysis. In development trial-7, the chromatographic condition was buffer (1.36g KH2PO4 in 1-liter water pH 7.0 with KOH). Mobile phase-A: 950 buffer, 50 methanol. Mobile phase-B: ACN, Water, OPA 950: 50: 1.75. Diluent: water, methanol 50:50 v/v. Inertsil ODS 3V 250 x 4.6mm,5µ, 1.0mL/min, 225nm, 30°C, 40µL and run time 90min. Gradient program: M.P-A at 0 min 100%, 10 min 100%, 75 min 76%, 80 min 70%, 85 min 70%, 90 min 10%, 100 min 10%, 105 min 100 % and 120 min 100%.CPM impurity a peak was separated with placebo peak at 41 min. In development trial-8, the chromatographic conditions were buffer (1.36g KH2PO4 in 1-liter water pH 4.0 with OPA). Mobile phase-A: buffer. Mobile phase-B: ACN, buffer 98: 2. Diluent: water, methanol 50:50 v/v. Inertsil ODS 3V 250mm, 4.6mm,5µ column, 0.7mL/min, 225nm, 30°C, 40µL and runtime 90min. Gradient program: M.P-A at 0 min 100%, 22 min 92%, 55 min 84%, 110 min 72%, 115 min 70%, 120 min 10%, 130 min 10%, 131 min 100 % and 155 min 100%. The process and degradation impurities of four active ingredients in flu cold tablet dosage form were well separated. Their purity angles were less than their purity thresholds in each stress condition. The development trial-8 gradient program was captured in **Table 2**. The system suitability chromatogram is shown in **Fig. 3.**

TABLE 2: GRADIENT PROGRAM FOR OPTIMIZED METHOD

Time (min)	M. Phase-A (%)	M. Phase-B (%)
0.01	100	0
22.0	92	8
55.0	84	16
110.0	72	28
115.0	70	30
120.0	10	90
130.0	10	90
131.0	100	0
155.0	100	0

This developmental trial conditions showing the separation between co-eluted peaks and unknown impurities were separated. The chromatogram represented all active components and known impurities mixed sample. The 60 °C degradation sample for 21 days was analyzed using this HPLC method, and results were found to be satisfactory. All peaks were separated, and peak shapes were also good. Eventually, these chromatographic conditions were finalized to determine the known and unknown impurities in the tablet dosage form.

Method Validation: The developed method was optimized and validated by following the ICH Q2 (R1) and USP general chapter for validation of an analytical procedure: 122524-27. The following validation parameters were performed.

Specificity: Specificity was performed to confirm the interference between diluent and placebo with product peaks, known and unknown impurities.

All known impurities and product peaks were well separated. Forced degradation studies were conducted to evaluate product degradation behaviour and impurities formation. Stress studies were conducted with 5N HCl and NaOH, 30% H_2O_2, thermal, water, UV light, 90% humidity. Stress study conditions and results were tabulated in **Table 3.**

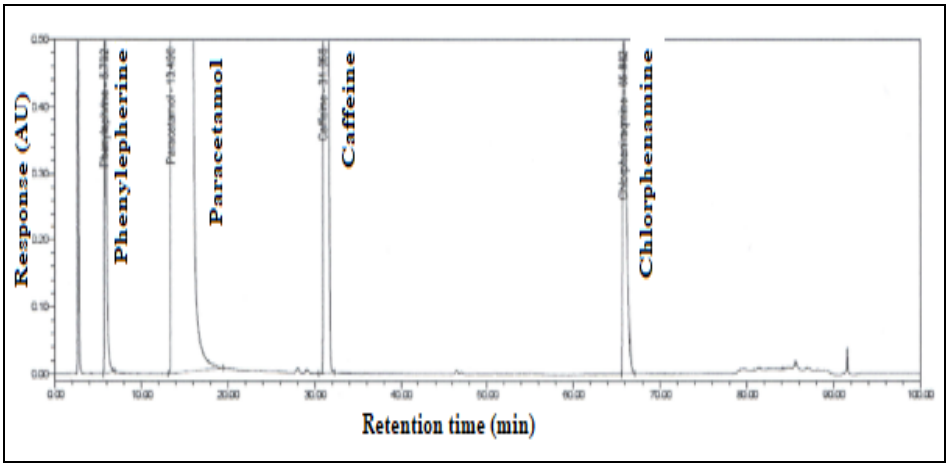

FIG. 3: STANDARD SOLUTION CHROMATOGRAM OF PA: PARACETAMOL, CA: CAFFEINE, PE: PHENYLEPHRINE, CP: CHLORPHENAMINE

Precision: Precision of the method was performed with six replicate test sample solutions with known impurities spiked with specification limit.

%RSD was calculated and found within the acceptable limit. Precision results were tabulated in **Table 4.**

Accuracy: Accuracy was performed with 50%, 100% and 150% concentration levels. % of recovery was calculated and found within the acceptable limit. Accuracy results were tabulated in **Table 4.**

Linearity: Linearity was performed from LOQ concentration to 150% of specification limit and correlation coefficient, a bias for 100% level, intercept value, slope value, residual sum of squares were calculated. A linearity plot was drawn between concentration and peak area. Linearity results were tabulated in **Table 4.**

TABLE 4: RESULTS OF VALIDATION (PRECISION, ACCURACY, LOD, LOQ AND LINEARITY)

Parameter	PE-C	PE-D	CPM-C	CPM-D
Method Precision (% RSD)	**0.1**	**0.2**	**0.2**	**4.1**
Linearity Correlation co-efficient	1.000	1.000	1.000	1.000
Y-intercept	705.158	-1421.3	1741.1	2422.1
Slope	29096.9	224606.0	109200.7	115043.6
Bias at 100 %	0.1	-0.3	1.2	1.6
R. S. Sq	134475503.9	6914046.8	872556.4	3977266.0
LOQ Precision (% RSD)	6.7	2.9	6.3	7.9

LOD and LOQ Establishment: Limit of detection (LOD) and Limit of quantification (LOQ) were established using visual and S/N ratio methods. Six replicate solutions were prepared at LOQ concentration and performed the precision for all four active components and known impurities. LOQ and LOQ results were tabulated in **Table 4.** The LOQ chromatogram is shown in **Fig. 4.**

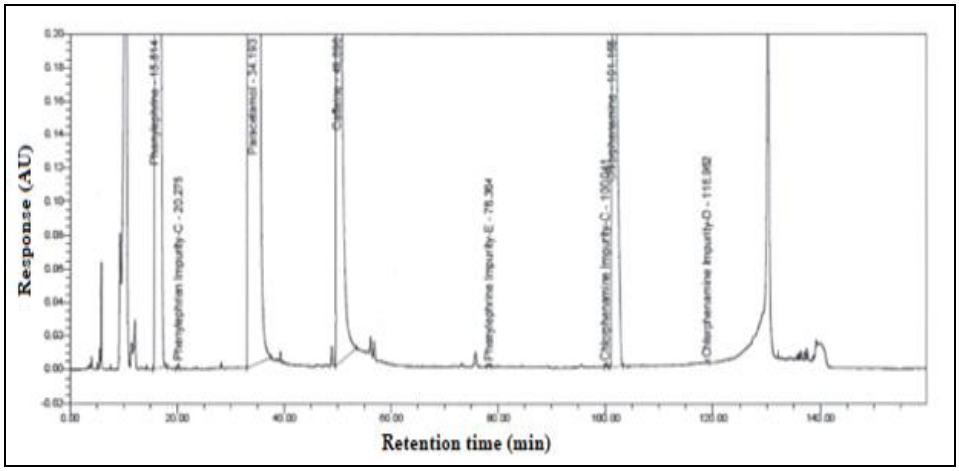

FIG. 4: LOQ CHROMATOGRAM OF KNOWN IMPURITIES IN THE PRESENCE OF FOUR ACTIVE PHARMACEUTICAL INGREDIENTS

Ruggedness and Robustness: Ruggedness was performed to confirm the system to system, column to column, and analyst to analyst difference. Solution stability studies were conducted for standard solution (benchtop day 0 to 6) and test solution (benchtop day 0 to 6). Robustness was conducted for flow rate (0.6mL/min to 0.8mL/min), column oven temperature (30°C to 40°C), and filter validation (PVDF, NYLON, and centrifuge). Results were confirmed the system suitability limits and differences between analysts, instruments, and columns. All variations and stability results have confirmed the robustness and ruggedness of the method.

CONCLUSION: Simple, accurate and stability-indicating HPLC method was developed and validated to determine the paracetamol, caffeine, chlorphenamine maleate, phenylephrine, and its impurities in flu cold combination dosage form. All related known and unknown impurities were well separated with good peak shape. Method validation was performed with precision, accuracy, linearity, ruggedness and robustness, specificity, LOD, and LOQ. The reported method is suitable to analyze the manufacturing products.

ACKNOWLEDGEMENT: The authors thank Dr. Reddy's Laboratories Ltd., Hyderabad, India, for these facilities to carry out this research. Support from colleagues of Analytical Research and process research of Dr. Reddy's Laboratories Ltd. is thankfully acknowledged.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Mukanova Z, Gudun K, Elemessova Z, Khamkhash L, Ralchenko E and Bukasov R: Detection of Paracetamol in Water and Urea in Artificial Urine with Gold Nanoparticle@ Al Foil Cost-efficient SERS Substrate. Analytical Sciences 2018; 34: 183-7.
2. Kharbuja K, Sharma M and Sharma NR: Comparative evaluation of effectiveness of intravenous paracetamol and intravenous diclofenac as post-operative analgesia in laparoscopic cholecystectomy. Journal of Lumbini Medical College 2018; 3: 6:6.
3. Balentine DA, Harbowy ME and Graham HN: Tea: The plant and its manufacture. Chemistry and Consumption of the Beverage 2019; 42-79.
4. Mürner-Lavanchy IM, Doyle LW, Schmidt B, Roberts RS, Asztalos EV, Costantini L, Davis PG, Dewey D, D Ilario J, Grunau RE and Moddemann D: Neurobehavioral outcomes 11 years after neonatal caffeine therapy for apnea of prematurity. Pediatrics 2018; 141: 20174047.
5. Curry C, Eldrup-Jorgensen J, Richard J, Siciliano MC and Craig WY: Phenylephrine infusion impact on surgical site infections after lower extremity bypasses surgery. Journal of Vascular Surgery 2018; 67: 287-93.
6. Wodack KH, Graessler MF, Nishimoto SA, Behem CR, Pinnschmidt HO, Punke MA, Monge-García MI, Trepte CJ and Reuter DA: Assessment of central hemodynamic effects of phenylephrine: an animal experiment. Journal of Clinical Monitoring and Computing 2018; 27: 1-8.
7. Sharma A, Thakur KK, Mehta P and Pathania D: Efficient adsorption of chlorpheniramine and hexavalent chromium (Cr (VI)) from water system using agronomic waste material. Sustainable Chemistry and Pharma 2018; 9: 1-1.
8. Ribeiro MM, Marra MC, Costa B, Oliveira TC, Batista AD, Muñoz RA and Richter EM: Sub-Minute method for determination of naphazoline in the presence of diphenhydramine, pheniramine or chlorpheniramine by capillary electrophoresis. Journal of the Brazilian Chemical Society 2018; 9: 1959-64.
9. Domínguez-Ramirez AM, López-Muñoz FJ, Medina JR, Hurtado M, Angeles GA, Pineda AD and Moreno-Rocha LA: HPLC-PDA method for the quantification of paracetamol in plasma: application to PK/PD studies with arthritic rats. Int J Pharm Pharm Sci 2017; 9: 233-9.
10. Aminu N, Chan SY, Khan NH, Farhan AB, Umar MN and Toh SM: A simple stability-indicating HPLC method for simultaneous analysis of paracetamol and caffeine and its

application to determinations in fixed-dose combination tablet dosage form. Acta Chromatographica 2018; 1-7.

11. Patel DK, Vyas AJ, Noolvi MN, Patel AB and Patel NK: Estimation of four drugs: Ambroxol hydrochloride, Levocetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol by RP-HPLC in tablet dosage form. International Journal of Pharmaceutical Chemistry and Analysis 2018; 5: 31-8.
12. Itodo AU and Onyinye NV: Comparative chromatographic and spectrophotometric methods for quantitative estimation of paracetamol in analgesic tablet dosage forms. Science Journal of Analytical Chemistry 2017; 5: 98.
13. Pahade AR, Gandhi SV and Tapale SR: Chemometric-assisted UV Spectrophotometric and RP-HPLC methods for the simultaneous determination of caffeine and sodium benzoate in synthetic mixture. Current trends in Biotechnology & Pharmacy 2017; 1: 11.
14. Finaru A and Elfakir C: Optimization of a HPLC analysis method for taurine and caffeine in energy drinks. Scientific study & research. Chemistry & chemical engineering, biotechnology. Food Industry 2018; 19: 23-32.
15. George J, Amalia M, Amelia TV and Bianca-Eugenia O: Determination of caffeine content in dietary supplements for weight loss by a HPLC-UV method. Acta Medica Marisiensis 2017; 2: 63.
16. Escobar SC, Cubides LR and Perez CP: Optimization and Validation of a simple and fast rp-hplc method for simultaneous determination of acetaminophen and caffeine in tablets. Indian Journal of Pharmaceutical Sciences 2017; 79: 731-9.
17. Zhang Z, Zhang Y and Gerk PM: Preparation of phenylephrine 3-O-sulfateasthe majorin-vivometabolite of phenylephrine to facilitate its pharmacokinetic and metabolism studies. Journal of Pharmacological and Toxicological Methods 2019; 95: 66-69.
18. Ragab MA, Abdel-Hay MH, Ahmed HM and Mohyeldin SM: Application of Capillary Zone Electrophoresis Coupledwitha DiodeArray Detector (CZE-DAD) to Simultaneous Analysisof Ibuprofen and Phenylephrine. Journal of AOAC Internationl 2019; 102(2): 473-479.
19. Ribeiro MM, Marra MC, Costa B, Oliveira TC, Batista AD, Munoz RA and Richter EM: Sub-Minute Method for Determination of Naphazoline in the Presence of Diphenhydramine, Pheniramine or Chlorpheniramine by Capillary Electrophoresis. Journal of the Brazilian Chemical Society 2018; 29: 1959-64.
20. Azmi SN, Al-Hadhrami SS, Al-Marhoubi BM, Al-Sulaimi SS and Al-Shamoosi ZD: Development and validation of fluorescence spectrophotometric method: quantitation of chlorpheniramine maleate in pharmaceutical formulations. Journal of Molecular Liquids 2017; 243: 750-60.
21. Ragab MA, El Yazbi FA, Hassan EM, Khamis EF and Hamdy MM: Stability studies of over the counter quaternary mixture containing phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol and caffeine using different chromatographic methods. Analytical Chemistry Letters 2018; 8: 331-47.
22. Zahoor A, Munir H, Junaid R, Hussain S, Naveed S, Alam MO, Khanum K, Qamar F and Khan S: A RP-HPLC method for simultaneous estimation of chlorpheniramine maleate, paracetamoland phenylephrine hydrochloride in bulk rads. Journal of Pharmacy and Pharmaceutical Sciences 2018; 18; 53-8.
23. Marzieh Rahimi, Neda Khorshidi and Rouhollah Heydari: Simultaneous determination of paracetamol and caffeine in aqueous samples by ultrasound assisted emulsification micro extraction coupled with highperformance liquid chromatography. Sep Sci Plus 2020; DOI.org/10.1002/sscp.202000069.
24. International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use ICH harmonised tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1), step 4 2005.
25. General Information/ (1225) Validation of Compendial Procedures. First Supplement to USP 43-NF 38, November 1 2019.
26. Deshpande A, Moses J Babu, Basavaiah K, Nagaraju Rajana, Ramana DV and Rama Rao Ganta: Dharamasoth Rama Devi4 Amol An orthogonal approach formethod development and validation of three potential halo alkyl alcohol genotoxic impurities inmiglitol drug substance by fast gas chromatography–mass spectrometry. Sep Sci plus.2020; DOI.org/10.1002/sscp.202000050.
27. Nagaraju Rajana, Ramana DV, Babu JM, Basavaiah K and Devi DR: Quantitative method for determination of 3, 3-dimethylallyl bromide genotoxic impurity in Tazarotene drug substance by GC-MS. Sep Sci Plus 2020; 1-9.

How to cite this article:
Rao GS, Devi DR, Reddy SP, Kalyanaraman L, Patel PKS, Subbaiah BV, Rao BM, Rajana N and Basavaiah K: Stability indicating HPLC method for paracetamol, caffeine, phenylepherine HCL, chlorphenamine male ate and its impurities in flucold tablet dosage form. Int J Pharm Sci & Res 2021; 12(12): 6424-31. doi: 10.13040/IJPSR.0975-8232.12(12).6424-31.