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Original Article

VALIDATED STABILITY INDICATING HPLC APPROACH FOR QUANTIFYING TRICHOLINE CITRATE AND CYPROHEPTADINE SIMULTANEOUSLY IN SYRUP FORMS

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ABSTRACT

Objective: This investigation demonstrates a stability-indicating and reliable "high-performance liquid chromatography" method to simultaneously quantify tricholine citrate (TEC) and cyproheptadine (CRH) in the syrup form and bulk form.

Methods: Successful separation was accomplished using C18 "Agilent column (250 mm × 4.6 mm, 5 µm)" with isocratic type of elution using mobile phase containing 0.1 M NaH_2PO_4 buffer and acetonitrile at 55% volume and 45% volume ratio, respectively with 1.0 ml/min flow rate. The wavelength sensor was attuned at 263 nm to quantify TEC and CRH.

Results: TEC and CRH peaks were eluted with fine resolution at retention times 1.837 min and 2.936 min, respectively. In the 137.5-412.5 µg/ml and 1-3 µg/ml concentration ranges for TEC and CRH, the calibration graphs were linear, with regression coefficients of 0.9999 and 0.9998, respectively. The suggested "high-performance liquid chromatography" approach has been shown as sensitive, precise, robust, accurate, specific and stability indicating through the resolution of TEC and CRH from its degradation-based compounds.

Conclusion: The established high-performance liquid chromatography technique was effectively extended to the evaluation of TEC and CRH in the combined syrup form and the test results appeared satisfactory.

Keywords: Tricholine citrate, Cyproheptadine, Syrup form, HPLC, Stability indicating

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INTRODUCTION

Tricholine Citrate (TEC) is recommended in the adults to treat and control hepatic and pancreatic disorders [1-3]. In hepatic cells, TEC confers lipotropic behaviour. TEC can also alleviate asthma complications and reduce the leukotriene pathway's pro-inflammatory and also inflammatory mediators. Chemically, TEC is described as 2-hydroxy-N,N,N-trimethylethanaminium 2-hydroxyproline-1,2,3-tricarboxylate (fig. 1). Cyproheptadine (CRH) relieves irritated, red, itchy, watery eyes, sneezing, and runny nose triggered by irritants present in air, allergies and hay fever [4-6]. CRH can often be used to relieve itching of allergic skin conditions and also to treat rashes, including rashes due to cold temperature stimulation and skin rubbing. CRH is in a category of medicines recognized as antihistamines. CRH functions by suppressing the activity of histamine, a material that triggers body's allergic reactions. Chemically CRH is described as 4-(5H-Dibenzo[a,d]cyclohepten-5-ylidene)-1-methylpiperidine (fig. 1).

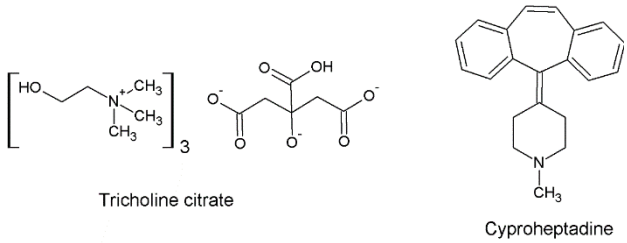

Fig. 1: Structures of drugs

Combination therapies become part of the standardized testing of all drugs and the treatment of all diseases [7-10]. A combination treatment is a therapeutic approach in which the patient is given more than one treatment. Cases of combination therapies include treatments that involve the treatment with many different medications, each comprising a specific drug, or the single medication containing several drugs.

The combination of TEC and CRH is available as a syrup formulation [11-15]. The TEC and CRH combination therapy increases appetite, offers the efficient hepato-protective operation, guarantees weight gaining and boosts protein synthesis. Combination treatment with TEC and CRH is prescribed for the hepatobiliary conditions linked with anorexia, leading to weight reduction [16]. To the maximum of our awareness, no procedures for measuring the combination of TEC and CRH in syrup preparations have been established yet. This paper proposes a novel sensitive stability-indicating HPLC procedure for the assessment of TEC and CRH combination. The process proposed enables the rapid assessment of the mixture of TEC and CRH in bulk drugs and syrup preparations without sample pretreatment with high precision and specificity, and with no excipient intervention.

MATERIALS AND METHODS

Chemicals

NaH_2PO_4, HCl, NaOH, H_2O_2 and orthophosphoric acid were acquired from "Sd Fine Chemicals Ltd, India". Acetonitrile was acquired from "Merck India Ltd, India". TEC and CRH bulk samples were gifted from "Rainbow pharma training labs, India". Cypro T formulation

syrup from "Nexkem Pharmaceutical, India" with labelled claim of 2 mg CRH and 275 mg TEC was utilized.

Instrument and evaluation conditions

Separations and analyses of TEC and CRH were accomplished using Waters chromatograph (2695) system furnished with Waters photodiode (2998) detector. The column employed was "Agilent C18 (250 mm × 4.6 mm and 5 µm)" with room temperature. Mobile phase employed was a combination of 0.1 M NaH_2PO_4 buffer and acetonitrile at 55% volume and 45% volume ratio, respectively with 1.0 ml/min flow rate. The buffer's pH was tuned at 4.0 value with 0.1% orthophosphoric acid. The mobile phase was degassed and filtered before utilization through degasser and 0.45 µm membrane filters, respectively. The peak area intensity of TEC and CRH was measured at 263 nm with the injection size volume of 10 µl.

Standard TEC and CRH solution

Standard TEC and CRH solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was prepared by dissolving 275 mg of TEC and 2 mg of CRH in 100 ml of mobile phase solvent blend. The standard TEC and CRH solution were diluted employing mobile phase solvent blend further as needed.

Procedures

Calibration graphs of TEC and CRH

Accurately assessed aliquot parts of the standard TEC and CRH were moved to a set of 10 ml volumetric bottles such that the ultimate concentrations were between 137.5-412.5 µg/ml (TEC) and 1-3 µg/ml (CRH). The solutions are finished to volume employing mobile phase solvent blend and blended well. Samples of 10 µl volume were infused and eluted under optimal chromatographic constraints with the mobile phase solvent blend. The analyte peak areas against the ultimate drug concentrations were plotted. The related regression equations for TEC and CRH were alternatively derived.

Analysis of TEC and CRH in the Cypro T formulation syrup

A measured volume (5 ml) of syrup equivalent to 2 mg CRH and 275 mg TEC was moved to 100 ml volumetric bottle, about 75 ml of mobile phase solvent blend was included and sonicated 30 min to dissolve TEC and CRH. The solution then was mobile phase-diluted to volume, blended and filtered. Aliquot consisting of concentration 2 µg/ml of CRH and 275 µg/ml of TEC was moved to a 10 ml volumetric bottle. The process described under "Calibration graphs of TEC and CRH" was executed. The nominal contents of TEC and CRH in Cypro T formulation syrup were evaluated either from related calibration graph or from related regression equation.

Procedures for TEC and CRH stability studies

In compliance with ICH recommendations, the stability studies on Cypro T formulation solution were done [17].

Degradation using alkaline

Volume (10 ml) of Cypro T formulation solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was moved to 100 ml volumetric bottle; 10 ml aliquot part of 0.1 N NaOH solution was included. The solution stayed sonicated 30 min at room temperature. After specified 30 min time, the contents of volumetric bottle were cooled, neutralized using 0.1N HCl. The solution then was mobile phase-diluted to volume, blended and filtered. The process described under "Calibration graphs of TEC and CRH" was executed. The assay and degradation percentage of TEC and CRH were evaluated.

Degradation using acid

Volume (10 ml) of Cypro T formulation solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was moved to 100 ml volumetric bottle; 10 ml aliquot part of 0.1 N HCl solution was included. The solution stayed sonicated 30 min at room temperature. After specified 30 min time, the contents of volumetric bottle were cooled, neutralized using 0.1N NaOH. The solution then was mobile phase-diluted to volume, blended and filtered. The process mentioned under "TEC and CRH calibration graphs" has been implemented. TEC and CRH were tested for the assay and degradation percentage.

Degradation using peroxide

Volume (10 ml) of Cypro T formulation solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was moved to 100 ml volumetric bottle; 10 ml aliquot part of 30% peroxide solution was included. The solution stayed sonicated 30 min at room temperature. After specified 30 min time, the solution then was mobile phase-diluted to volume, blended and filtered. The process described under "Calibration graphs of TEC and CRH" was executed. The assay and degradation percentage of TEC and CRH were evaluated.

Degradation using sun light

Volume (10 ml) of Cypro T formulation solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was moved to 100 ml volumetric bottle. The bottle with Cypro T formulation solution was lay open to sun light for a time of 6 hr. After specified 6 hr time, the solution then was mobile phase-diluted to volume, blended and filtered. The process described under "Calibration graphs of TEC and CRH" was executed and followed by evaluation of assay and degradation percentage of TEC and CRH.

Degradation using dry heat

Volume (10 ml) of Cypro T formulation solution containing 2750 µg/ml (TEC) and 20 µg/ml (CRH) was moved to 100 ml volumetric bottle. The bottle with Cypro T formulation solution was wide-open to 60 °C in oven for a time of 30 min. After specified 30 min time, the solution then was mobile phase-diluted to volume, blended and filtered. The assay and degradation percentage of TEC and CRH were evaluated employing process explained under "Calibration graphs of TEC and CRH".

RESULTS

Validation of procedure

In acquiescence with ICH recommendations, the validity parameters were established [18].

Linearity

During this work, the linearity of area response was checked for both TEC and CRH. Chromatographed solutions with concentrations of 137.5-412.5 µg/ml for TEC and 1-3 µg/ml for CRH given linear peak response areas. The regression line equation, regression coefficient and TEC and CRH calibration curves are shown in fig. 2.

Limits of quantification (Lq) and detection (Ld)

Both Lq and Ld were measured utilizing a signal-to-noise methodology. Lq and Ld were defined as the TEC an CRH concentration levels that ensuing a peak height of 10 times and 3 times, respectively the baseline noise. The Ld and Lq were found to be 0.124 µg/ml and 0.413 µg/ml, respectively, for TEC and 0.0023 µg/ml and 0.0078 µg/ml, respectively, for CRH.

Accuracy and precision

The accuracy and precision measurements were assessed using measurements of TEC and CRH solution (2 µg/ml and 275 µg/ml) repeated six times within the day. The precision was validated by the RSD measurements of the TEC and CRH peak areas, while the accuracy was validated by the TEC and CRH percentage content assays (table 1).

Recovery

The recovery was determined by assay of TEC and CRH in spiked Cypro T samples according to proposed method. Three diverse quantities (50% quantity degree, 100% quantity degree and 150% quantity degree) of TEC and CRH standards were put into Cypro T samples. The recovered values of TEC and CRH were presented in table 2.

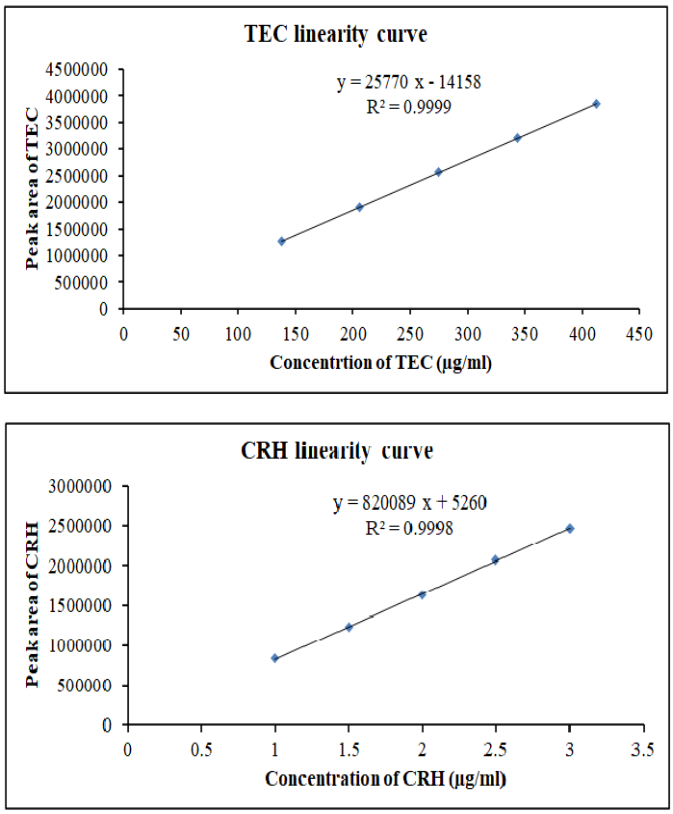

Fig. 2: TEC and CRH linearity curves

Table 1: Precision and accuracy measurements for TEC and CRH

Sample No.	Precision		Accuracy	
	TEC area	CRH area	TEC % assay	CRH % assay
1	2581692	1673134	99.48	100.60
2	2586089	1673918	99.65	100.65
3	2610132	1680554	100.58	101.05
4	2599406	1661186	100.17	99.88
5	2595038	1664656	100.00	100.09
6	2587896	1680666	99.72	101.05
Avg.	2593375.50	1672352.33	99.93	100.55
S. D	10387.8131	8041.9805	0.4025	0.4843
R. S. D	0.4006	0.4809	0.4027	0.4817

Avg.–mean of six measurements; SD–Deviation of value for six measurements; RSD–Percentage deviation

Table 2: Recoveries of TEC and CRH

Spiked degree (%)	Quantity added (µg/ml)	Quantity quantified* (µg/ml)	Quantity recovered* (%)	SD	RSD
50	136.125	134.90	99.10	0.4912	0.496
100	272.2	271.88	99.86	0.1815	0.182
150	408.375	408.88	100.12	0.0702	0.070
50	0.99	0.99	100.42	0.0656	0.065
100	1.98	1.97	99.53	0.3932	0.395
150	2.97	2.96	99.71	0.1015	0.102

*mean of three measurements; SD–Deviation of value for three measurements; RSD–Percentage deviation

Robustness

The robustness was measured using peak area measurements of TEC and CRH solution (2 µg/ml and 275 µg/ml) with considerably changed parameters in HPLC assay operating conditions. The changed parameters and peak areas obtained were presented in table 3.

Selectivity

To prove selectivity, a comparison of blank (mobile phase solvent blend), TEC and CRH solution (2 µg/ml and 275 µg/ml) and Cypro T sample (2 µg/ml and 275 µg/ml) chromatograms were made. The respective chromatograms were given away in fig. 3.

Table 3: Robustness of assay of TEC and CRH

Parameter	Condition employed	TEC			CRH		
		Peak area	SD	RSD	Peak area	SD	RSD
Acetonitrile volume (%)	40	2598351	40645.7451	1.585	1682281	30050.9462	1.816
	45	2576531			1659686		
	50	2519624			1622752		
Flow rate (ml/min)	0.9	2524231	42473.4756	1.653	1620685	31160.0151	1.884
	1.0	2576531			1659686		
	1.1	2608351			1682281		
pH	3.8	2573576	2122.9900	0.082	1653039	3565.5242	0.215
	4.0	2576531			1659686		
	4.2	2572413			1654126		
Wavelength (nm)	261	2503577	36537.1568	1.439	1685964	31122.2638	1.879
	263	2576531			1659686		
	265	2536424			1623959		
Temperature (°C)	23	2539624	29307.7494	1.140	1632752	23092.1729	1.394
	25	2576531			1659686		
	27	2597514			1678710		

SD–Deviation of value for three peak area measurements; RSD–Percentage deviation

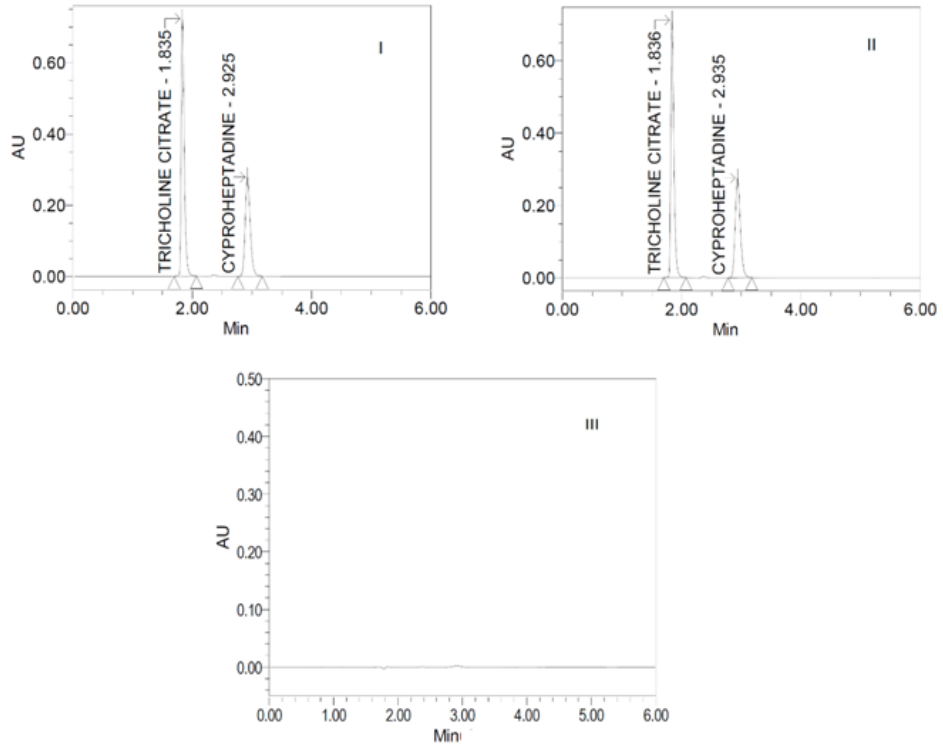

Fig. 3: Obtained chromatograms for (I) Cypro T sample (II) TEC an CRH solution (III) Blank

Degradation study and specificity

These investigations have been completed to explicate the intrinsic stability capabilities of TEC and CRH in combination under distinct situations of forced degradation described earlier. By using 0.1 N HCl, 30% peroxide and dry heat, TEC percent recovery was decreased about 11.69%, 6.74% and 7.32%, respectively while CRH percent recovery was decreased about 8.42%, 4.97% and 6.52%, respectively. The chromatograms corresponding to TEC and CRH in 0.1N HCl, 30% peroxide and dry heat degradation were shown in fig. 4. I, 4. II and 4. III. In sunlight and 0.1 N NaOH conditions, TRH percent recovery was decreased about 9.08% and 5.36%, respectively and CRH was decreased by about 10.94% and 7.77%, respectively. The chromatograms corresponding to TEC and CRH in sunlight and 0.1 N NaOH degradation conditions were displayed in fig. 4. IV and 4. V. The additional peaks generated, in the course of 0.1 N HCl, 30% peroxide, dry heat, sunlight and 0.1 N NaOH conditions applied, resolved finely from the TEC and CRH peaks (fig. 4. I to 4. V).

System suitability

The system suitability measurements were assessed using measurements of TEC and CRH solution (2 µg/ml and 275 µg/ml) repeated five times. The system suitability was validated by measurements of plate counts, tailing symmetry, resolution, retention time and peak areas for the TEC and CRH peak (table 4).

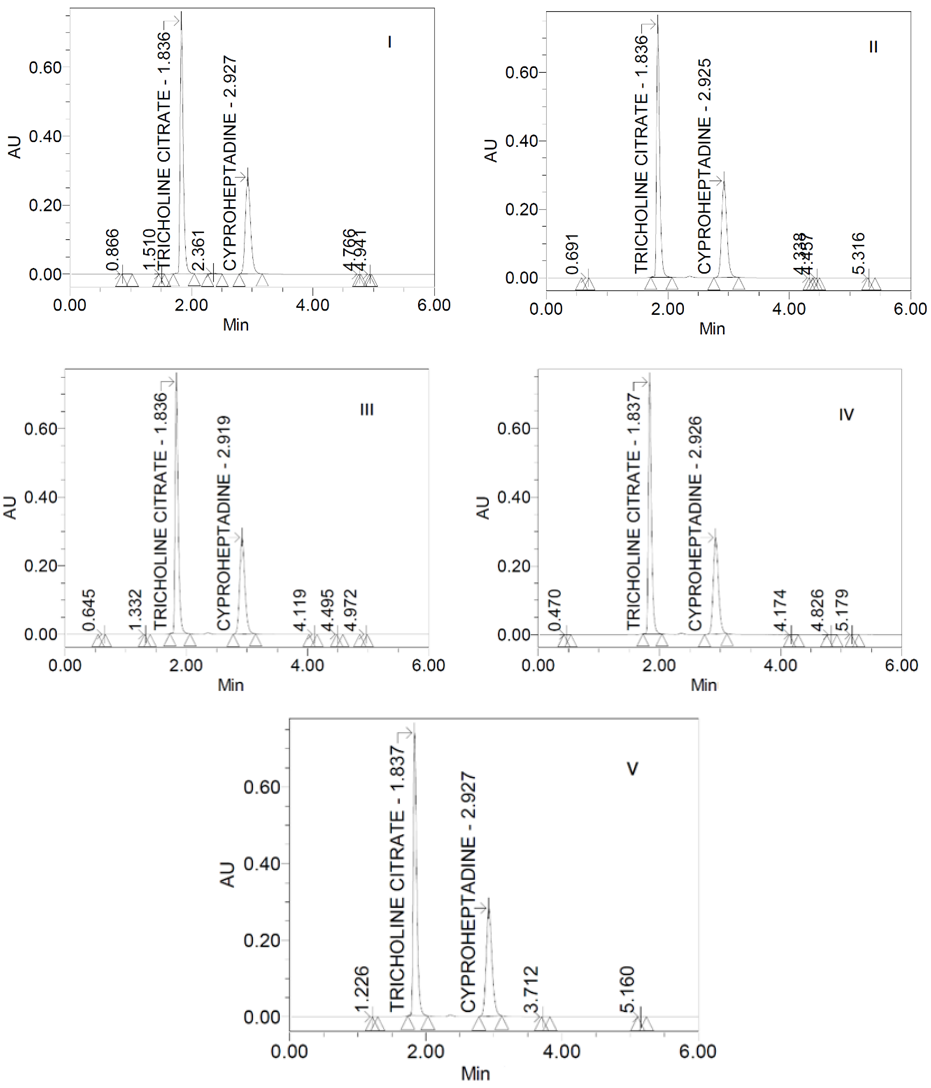

Fig. 4: Obtained chromatograms for degradation with (I) 0.1N HCl (II) 30% peroxide (III) Dr heat (IV) Sun light (V) 0.1N NaOH

Table 4: System suitability for assay of TEC and CRH

Statistical data	Retention period (min)	Area	Resolution	Plate count	Tailing
	TEC				
Avg.	1.837	2582140	-	6173	1.488
SD	0.0011	8718.8306	-	125.6523	0.0130
RSD	0.060	0.338	-	2.036	0.876
	CRH				
Avg.	2.936	1656492	8.560	5625	1.196
SD	0.0020	5194.4251	0.0361	39.4614	0.0089
RSD	0.068	0.314	0.421	0.702	0.748

Avg.–mean of five measurements; SD–Deviation of value for five measurements; RSD–Percentage deviation

DISCUSSION

To quantify CRH methods like spectrofluorimetry (by Belal *et al.*,) [19], UV spectrophotometry (by Wagh *et al.*,) [20], colorimetry (by Sayanna *et al.*,) [21], HPLC (by Maham *et al.*, Sadhana *et al.*, Mina *et al.*, El-Din *et al.*,) [22-25] and LC-MS (Feás *et al.*,) [26] were reported. To quantity TEC no reports were found. Similarly, no procedures for measuring the combination of TEC and CRH in syrup preparations have been established yet. This paper proposes a novel sensitive stability-indicating HPLC procedure for the assessment of TEC and CRH combination.

At the time of developmental trials, different C18 columns like YMC, Xterra, Waters and Agilent were investigated. The YMC and Xterra have dimensions of 150 mm length, 4.6 mm identification and 5 µm particle size. The Waters and Agilent have dimensions of 250 mm length, 4.6 mm identification and 5 µm particle size. As a mobile phase solvent blend, various solvent blends of different proportions have been checked. They include 0.1M K_2HPO_4: methanol, 0.1M Na_2HPO_4: acetonitrile and 0.1M NaH_2PO_4: acetonitrile. Resolution of TEC and CRH peaks was acceptable with C18 Aligent column at ambient temperature. 0.1M NaH_2PO_4 with 4 pH value and acetonitrile at the proportion of 55% volume and 45% volume [23], respectively, were optimized for the finest TEC and CRH peak shape and less retention time [23-26] for this proposed chromatographic process. To rapidly separate TEC and CRH, an isocratic elution mode

with 1.0 ml/min flow speed was preferred. At 263 nm, the response of the TEC and CRH was greater. Therefore, 263 nm was deemed an appropriate wavelength for TEC and CRH quantification.

In part due to linear values, the linearity of calibration displays for TEC and CRH was verified by the large regression coefficient (R^2) values for the TEC and CRH [18, 27, 28]. The Ld values and Lq values of TEC and CRH met the sensitivity prerequisite for quantifiable analysis of TEC and CRH [19-21, 25-28]. The RSD measurements and percentage content assays of TEC and CRH confirmed the good preciseness and accurateness, respectively, of HPLC method proposed [19-26]. The recoveries of TEC and CRH validated the accuracy and selectivity of HPLC method proposed [18, 27, 28]. When modifications to the robustness analysis were made, no major differences were noted. The robustness of the suggested HPLC approach is thus proven [18, 27,28].

The percentile degradation specified that the TEC is really sensitive than CRH to 0.1 N HCl, 30% peroxide and dry heat degradation conditions executed. The stability of TRH is less than that of CRH under the sunlight and 0.1 N NaOH degradation conditions executed as the percent recovery of TRH was less than CRH. Complete resolution of TEC and CRH peaks from its degradation-based compounds with no seeming shoulders confirmed the stability indicating feature and specificity of the suggested HPLC approach [17, 29-31].

Blank chromatogram showed no any peaks at retention times of TEC and CRH. While peak retention times for TEC and CRH was almost similar in the Cypro T sample chromatogram and TEC and CRH solution chromatogram. Thus, suggested HPLC approach selectivity was verified [18-26]. The system suitability measurements like plate counts, tailing symmetry, resolution, retention time and peak areas for the TEC and CRH peak fall within the ICH prescribed criterion, indicating appropriateness of HPLC instrumentation for assay of TEC and CRH combination by suggested approach [18, 27, 28].

CONCLUSION

A “high-performance liquid chromatography” process for determining the combination of TEC and CRH in the syrup form and pure form has been described in the established method. The present “high-performance liquid chromatography” process is exemplified by its speed, ease and relatively inexpensive. The successful validity criteria of the proposed approach permit its use in laboratories for quality control.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Nil

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