Talanta 79 (2009) 1360–1367

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms

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ARTICLE INFO

Article history:
Received 22 March 2009
Received in revised form 30 May 2009
Accepted 1 June 2009
Available online 9 June 2009

Keywords:
Paracetamol
Dantrolene
Cetirizine
Pseudoephedrine
Reversed-phase HPLC
Stability

ABSTRACT

A stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method has been developed which can separate and accurately quantitate paracetamol, dantrolene, cetirizine and pseudoephedrine. The method was successfully validated for the purpose of conducting stability studies of the four analytes in quality control (QC) laboratories. The stability-indicating capability of the method was demonstrated by adequate separation of these four analytes from all the degradant peaks. A gradient mobile phase system consisting of (A) 50 mmol L^{-1} sodium dihydrogen phosphate, 5 mmol L^{-1} heptane sulfonic acid sodium salt, pH 4.2 and (B) acetonitrile was used with Discovery reversed-phase HS C_{18} analytical column (250 mm × 4.6 mm i.d., 5 μm particle size). Quantitation was achieved with UV detection at 214 nm, based on peak area.

The proposed method was validated and successfully applied for the analysis of pharmaceutical formulations and laboratory-prepared mixtures containing the two multicomponent combinations.

1. Introduction

Most cold medicines contain multiple active ingredients that include antipyretics, analgesics, antitussive agents, mucolytic agents, bronchodilators, antihistamines, and several vitamins. Combinations of these compounds were analyzed using RP-HPLC procedures. However, as it is difficult to analyze simultaneously many different kinds of ingredients using a single method, ingredients were often divided into several groups based on their chemical properties, i.e. cationic compounds in one run and anionic or neutral compounds in another run. Gradient elution was also required for simultaneous analyses [1].

Analgesics such as paracetamol are widely used drugs, not only as pain relievers but also in several diseases (musculoskeletal and joint disorders, rheumatic disorders, arthritis, and rheumatism) [2]. Their determination in pharmaceutical dosage forms (quality control) remains of great interest. Among the various analytical techniques, high-performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of analgesic drugs and related compounds.

Paracetamol (PR), a para-aminophenol derivative, has analgesic and antipyretic properties and weak anti-inflammatory activity [2]. Dantrolene sodium (DT) is a muscle relaxant with a direct action on skeletal muscle. It uncouples muscular contraction from excitation, probably by interfering with the release of calcium from the sarcoplasmic reticulum [2]. Cetirizine hydrochloride (CT), a piperazine derivative and metabolite of hydroxyzine, described as a long-acting non-sedating antihistamine with some mast-cell stabilizing activity. It appears to have a low potential for drowsiness in usual doses and to be virtually free of antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria [2]. Pseudoephedrine (PS) is a direct- and indirect-acting sympathomimetic. It is a stereoisomer of ephedrine and has a similar action, but has been stated to have less pressor activity and fewer CNS effects. Pseudoephedrine and its salts are given by mouth for the symptomatic relief of nasal congestion. They are commonly combined with other ingredients in preparations intended for the relief of cough and cold symptoms [2]. The development of new pharmaceutical forms with classical active compounds generates new analytical problems. That is the case of capsules containing PR with DT (mixture 1) or CT and PS (mixture 2).

Several HPLC methods have been reported in literature for the determination of PR with CT [3,4], PR with PS [5–20], CT with PS [21–25] and DT alone [26–31]. Other methods have been reported; capillary electrophoresis [32,33], and TLC [34,35] have been used to determine some of these compounds in their preparations.

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doi:10.1016/j.talanta.2009.06.003

However, thorough literature search revealed no report of any stability-indicating analytical method for simultaneous determination of PR and DT (mixture 1); or PR, CT and PS (mixture 2) as multicomponent preparations.

Forced degradation has to demonstrate specificity when developing stability-indicating methods and for this reason, it should be performed prior to implementation of stability studies to assure that analytical methods are stability-indicating [36].

Therefore, there is a challenge to develop a stability-indicating RP-HPLC method for PR, DT, CT and PS. The challenge is to obtain a separation of PR, DT, CT and PS from each other and from a huge number of degradant peaks. Hence an attempt has been made to develop a sensitive, accurate, linear, precise, reproducible, repeatable, specific and robust analytical method for the determination of PR, DT, CT and PS in the presence of their degradants and also capable to separate all the major degradant peaks from each other.

Therefore, the present study was involved in a research effort aimed at developing and validating a simple, specific, accurate and precise new stability-indicating HPLC method for simultaneous determination of PR, DT, CT and PS for use in stability studies and quality control applications.

2. Experimental

2.1. Instrumentation

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μL loop and a SPD-10AVP UV–vis detector. Separation and quantitation were made on a Discovery reversed-phase HS C_{18} analytical column (250 mm × 4.6 mm i.d., 5 μm particle size, Sigma–Aldrich, USA). The detector was set at λ 214 nm for (mixture 1 and mixture 2). Class-VP software performed data acquisition.

2.2. Materials and reagents

Pharmaceutical grade of PR, DT, CT and PS were used and certified to contain 99.7, 99.4, 99.5, and 99.8%, respectively.

Acetonitrile used was HPLC grade (Sigma–Aldrich chemie GmbH D-8955 Steinheim, Germany) and methanol was HPLC grade (LabScan 44-101 Gliwice, ul.Sowinskiego 11, Poland). Heptanesulfonic acid sodium salt (Acros Organics, New Jersy, USA). Sodium dihydrogen phosphate and hydrochloric acid (Riedel-de Haën Laboratory Chemicals, Germany). Sodium hydroxide, 30% hydrogen peroxide (Sigma–Aldrich chemie GmbH D-8955 Steinheim, Germany) and phosphoric acid (BDH Laboratory Supplies Poole, England) used were analytical grade.

Alercet Cold® capsule (batch no. 820815) (Global Napi Pharmaceuticals, 6th October, Giza, Egypt), contains 400 mg of PR, 30 mg PS as HCl salt and 10 mg of CT as HCl salt per capsule; and Dantrelax Compound® capsule (batch no. 80077A) (Chemipharm Pharmaceutical Industries, 6th October, Giza, Egypt), contains 300 mg of PR and 25 mg of DT as sodium salt per capsule were used.

2.3. HPLC method

The HPLC separation and quantitation were made on Discovery reversed-phase HS C_{18} analytical column (250 mm × 4.6 mm i.d., 5 μm particle size) for mixture 1 and mixture 2. A gradient mobile phase system consisting of (A) 50 mmol L^{-1} sodium dihydrogen phosphate, 5 mmol L^{-1} heptane sulfonic acid, pH 4.2 and (B) acetonitrile was used.

The separation was achieved with a gradient program consisting of 0–3.5 min 15% mobile phase B and 3.5–8 min gradient up to 38% mobile phase B, and 8–22 min gradient up to 45% mobile phase B and 22–30 min gradient up to 50%. After 30 min the gradient was returned to the initial conditions and the analytical column was reconditioned for 3 min. The flow rate was 1.5 mL min^{-1} for mixture 1 and mixture 2. All determinations were performed at ambient temperature. The injection volume was 20 μL. The detector was set at λ 214 nm. Data acquisition was performed on class-VP software.

2.3.1. Standard solutions and calibration

Stock standard solutions of PR, DT, CT and PS were prepared separately by dissolving 50 mg of each compound in 50 mL methanol.

2.3.2. Calibration of HPLC method

The standard solutions were prepared by further dilutions of the stock standard solutions with the specified mobile phase to reach the concentration range of 2–115 μg mL^{-1} for PR, 0.5–50 μg mL^{-1} for DT, 0.5–50 μg mL^{-1} for CT and 1–50 μg mL^{-1} for PS.

Triplicate 20 μL injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations.

2.4. Forced degradation conditions

In general, to determine whether the analytical method was stability-indicating assay, forced degradation studies under different conditions were carried out on standards and excipients (starch, aerosil, talc and magnesium stearate) solutions according to the following conditions:

(a) Acidic and basic conditions: Solutions were treated with 2 mL of 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH. The flasks were placed in a thermostatic oven at 80 °C for 12 h, and then neutralized by adjusting the pH to 7.0.
(b) Oxidation with H_2O_2: Solutions were treated with 0.4 mL of hydrogen peroxide 30% (w/v). The flasks were placed in a thermostatic oven at 80 °C for 12 h.
(c) Solution stability: Solutions were protected from light and kept at ambient temperature for 48 h in order to observe short-term stability of the stock solutions.

Once the different treatments were complete, all the solutions and blanks (solutions without any treatment) were filtered with a 0.45 μm syringe filtration disk to the vials for injection in the HPLC system.

2.5. Sample preparation

2.5.1. For mixture 1

The contents of 20 hard capsules were emptied, weighed and mixed. An accurately weighed portion of the powder equivalent to 96 mg PR and 8 mg DT was extracted and diluted to 100 mL with methanol. The sample solution was filtered. Further dilution was carried out with the mobile phase to reach the calibration range of each compound. The general procedures for HPLC method described under calibration were followed and the concentrations of PR and DT were calculated.

2.5.2. For mixture 2

The contents of 20 hard capsules were emptied, weighed and mixed. An accurately weighed portion of the powder equivalent to 100 mg PR, 2.5 mg CT, and 7.5 mg PS was extracted and diluted to 100 mL with methanol. The sample solution was filtered. Further dilution was carried out with the mobile phase to reach the calibration range of each compound. The general procedures for

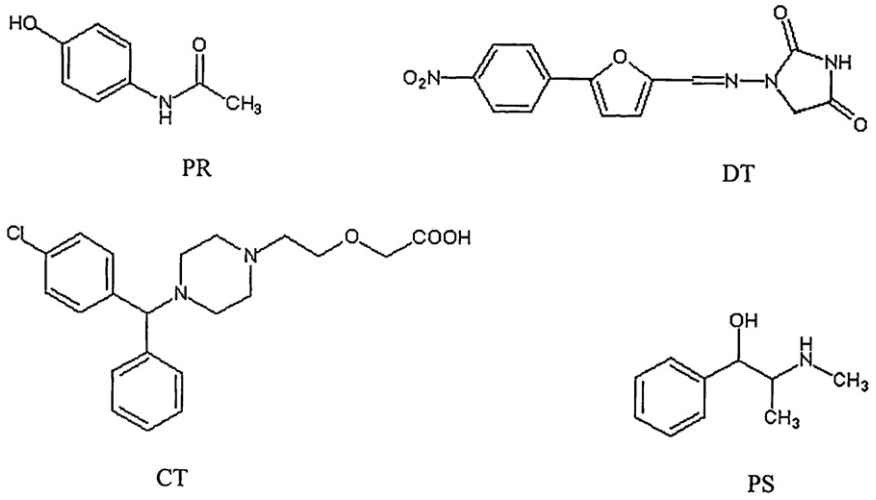

Fig. 1. Chemical structures of PR, DT, CT and PS.

HPLC method described under calibration were followed and the concentrations of PR, CT and PS were calculated.

3. Results and discussion

Fig. 1 shows the chemical structures of PR, DT, CT and PS.

3.1. HPLC method

During the optimization of the method three pH values (3.0, 4.2 and 5.0) with and without heptanesulfonic acid sodium salt (HAS) as ion pairing, and two organic solvents (methanol and acetonitrile) were tested.

The concomitant effects of optimum eluent composition, pH and sodium dihydrogen phosphate concentration for the determination of PR, DT, CT and PS in the presence of their degradants by HPLC were studied. The preliminary studies were carried out by the injection of a sample solution containing PR, DT, CT and PS.

In the HPLC system, mobile phase consisted of two different solvents: acetonitrile (A), and sodium dihydrogen phosphate (B). For these preliminary experiments, a 10 mmol L^{-1} sodium dihydrogen phosphate, pH 4.2 was employed. Flow rate of mobile phase was 1.5 mL min^{-1}.

The preliminary experiments shown bad resolution between PR, PS and other polar degradants, so formation of its ion pair with HSA was tried; as HSA was added to improve the separation, peak shape, symmetry of CT and DT and increase k' for early eluting compounds (PR and PS), but this resulted in very late elution for CT and DT. Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible gradient elution instead of isocratic elution, as gradient elution will enhance the separation between the analytes and their degradants in shorter time.

After trying several mobile phases containing acetonitrile or methanol with various buffers, the one consisting of (A) 50 mmol L^{-1} sodium dihydrogen phosphate, 5 mmol L^{-1} heptane sulfonic acid, pH 4.2 and (B) acetonitrile proved to be useful for separation between analytes and their degradants.

In relation with the organic solvent, acetonitrile provided better baseline at 214 nm and lower column backpressure than methanol.

This low wavelength was necessary to get enough sensitivity for the low concentration of PS in its pharmaceutical formulation.

pH variation of the 50 mmol L^{-1} sodium dihydrogen phosphate has a small considerable effect on the chromatographic behavior of PS and CT. At pH 3, CT (containing acidic group) retention time was slightly increased as an acidic compound is difficultly eluted in their un-ionised form. At pH 5, PS retention time was slightly decreased but the peak was tailed and coelution of CT with one of its degradants was occurred. However, at pH 4.2, optimum resolution with reasonable retention time was observed.

The influence of the sodium dihydrogen phosphate concentration of the mobile phase was studied by changing it from 10 to 100 mmol L^{-1} in the solution. The sodium dihydrogen phosphate concentration (50 mmol L^{-1}) was selected because it gives better peak shape for CT and DT.

The optimum concentration of HSA was chosen to be 5 mmol L^{-1} as it gives better peak shape and symmetry for the analytes.

Based on these investigations, good chromatographic separation between PR, DT, CT and PS in the presence of their degradant products was achieved by the use of a gradient mobile phase system consisting of (A) 50 mmol L^{-1} sodium dihydrogen phosphate, 5 mmol L^{-1} heptane sulfonic acid, pH 4.2 and (B) acetonitrile.

The separation was achieved with a gradient program consisting of 0–3.5 min 15% mobile phase B and 3.5–8 min gradient up to 38% mobile phase B, and 8–22 min gradient up to 45% mobile phase B and 22–30 min gradient up to 50%. After 30 min the gradient was returned to the initial conditions and the analytical column was reconditioned for 3 min. However, the quite long run time of this method can be overcome by the use of ultra high pressure liquid chromatography (UHPLC). As the linear velocity of the UHPLC column leads to much shorter chromatographic run times for similar separations". But unfortunately UHPLC is not available in our laboratory.

The specificity of the HPLC method is illustrated in Figs. 2–4 where complete separation of the studied compounds was noticed. The average retention time ± standard deviation for PR, DT, CT and PS were found to be 2.68 ± 0.04, 8.93 ± 0.07, 12.26 ± 0.02 and 13.72 ± 0.03 min, respectively, for 10 replicates. The system suitability test results of the developed method are presented in Table 1.

3.2. Degradation behavior

HPLC studies on PR, DT, CT and PS under different stress conditions indicated that:

PS, DT and excipients were stable under acidic conditions, but PR and CT were degraded. PR degraded to give PRD_1 peak at retention time 1.8 min. CT degraded to give CTD_2 peak at retention time 18.01.
PS, CT and excipients were stable under alkaline conditions, but PR and DT were degraded. PR degraded to give PRD_1 peak at 1.8 min.

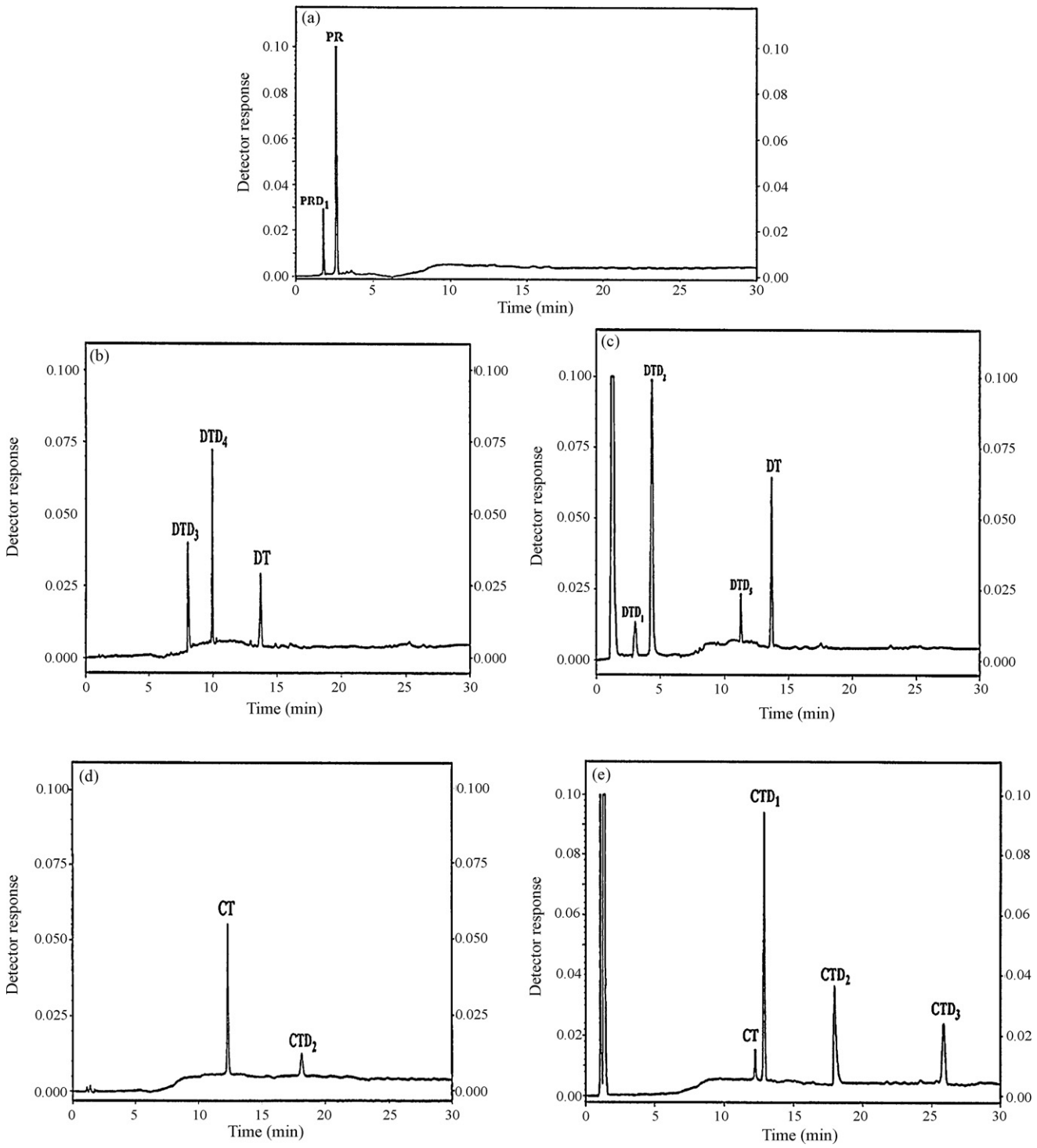

Fig. 2. HPLC chromatogram of 20 μL injection of (a) PR degradation in HCl, (b) DT degradation in NaOH, (c) DT degradation in H_2O_2, (d) CT degradation in HCl, and (e) CT degradation in H_2O_2.

DT degraded to give DTD_3 and DTD_4 peaks at 8.06 and 9.94 min, respectively.

PR, PS and excipients were stable under oxidative conditions, but DT and CT were degraded. DT degraded to give DTD_1, DTD_2, and DTD_5 peaks at 3.11, 4.31, and 11.28 min, respectively. CT was degraded to give CTD_1, CTD_2, and CTD_3 peaks at 12.91 and 18.04, 25.96 min, respectively.

PR, DT, CT, PS and excipients were stable at ambient temperature for 48 h.

3.3. System suitability

System suitability test parameters must be checked to ensure that the system is working correctly during the analysis. Method performance data including capacity factor (k'), selectivity (α), resolution (R_S), and tailing factor are listed in Table 1. All were satisfactory and indicative of the good specificity of the method for assessment of the stability of PR, DT, CT and PS.

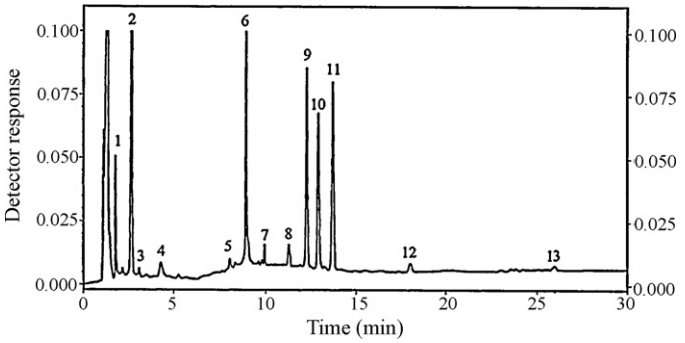

Fig. 3. HPLC chromatogram of 20 μL injection of laboratory-prepared mixture of PR, DT, CT, and PS and their degradants (1-PRD_1, 2-PR, 3-DTD_1, 4-DTD_2, 5-DTD_3, 6-PS, 7-DTD_4, 8-DTD_5, 9-CT, 10-CTD_1, 11-DT, 12-CTD_2, 13-CTD_3).

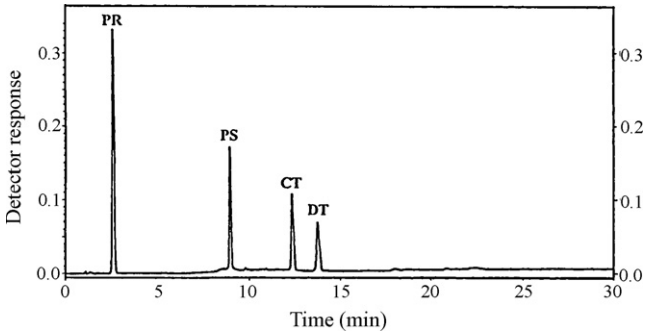

Fig. 4. HPLC chromatogram of 20 μL injection of laboratory-prepared mixture of 100 μg mL^{-1} of PR, 50 μg mL^{-1} of PS, 50 μg mL^{-1} of CT, and 50 μg mL^{-1} of DT.

3.4. Analysis of pharmaceutical products

The proposed HPLC method was applied to the simultaneous determination of PR and DT in Dantrelax Compound® capsule and PR, CT and PS in Alercet Cold® capsule. Determination of seven replicates was made. Satisfactory results were obtained for each compound in good agreement with label claims (Tables 2 and 3).

3.5. Validation of the methods

3.5.1. Linearity

The linearity of the HPLC method for determination of PR, DT, CT and PS was evaluated by analyzing a series of different concentrations of each drug. In this study seven concentrations were chosen, ranging between 2 and 115 μg mL^{-1} for PR, 0.5 and 50 μg mL^{-1} for DT, 0.5 and 50 μg mL^{-1} for CT and 1 and 50 μg mL^{-1} for PS. Each concentration was repeated three times; in order to provide information on the variation in peak area values between samples of same concentration. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($P = 0.05$) different from zero (Table 4). Characteristic parameters for regression equations of the HPLC method obtained by least squares treatment of the results are given in Table 4.

Upon Analytical Methods Committee (AMC), a value of regression coefficient close to unity is not necessarily the outcome of a linear relationship and in consequence the test for the Lack of Fit should be checked [37] (Table 5). This test evaluates the variance of the residual values [38]. The calculated values were lower than the tabulated ones ($\alpha = 0.05$) and linearity thus being demonstrated.

3.5.2. Precision

In order to judge the quality of the elaborated method precision was determined. For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each compound. The data for each concentration level was evaluated by one-way ANOVA. An 8 days × 2 replicates design was performed. Statistical comparison of the

Table 2
Determination of PR, CT, and PS in Alercet Cold® capsule using the proposed HPLC method.

Sample no.	Concentration (μg mL^{-1})			% recovery		
	PR	PS	CT	PR	PS	CT
1	20	1.5	0.5	99.7	100.9	101.3
2	40	3	1	99.5	100.6	101.1
3	60	4.5	1.5	100.2	100.5	99.5
4	72	5.4	1.8	100.4	99.8	100.3
5	80	6	2	99.6	100.7	99.1
6	100	7.5	2.5	100.2	99.4	100.5
7	112	8.4	2.8	99.8	99.6	100.7
Mean				99.91	100.21	100.36
S.D.				0.35	0.60	0.81

Table 3
Determination of PR, and DT in Dantrelax Compound® capsule using the proposed HPLC method.

Sample no.	Concentration (μg mL^{-1})		% recovery	
	PR	DT	PR	DT
1	12	1	100.7	100.9
2	24	2	99.6	101.1
3	36	3	100.5	99.2
4	48	4	99.5	99.7
5	60	5	99.6	100.6
6	84	7	99.8	99.6
7	108	9	99.9	99.5
Mean			99.94	100.09
S.D.			0.47	0.76

Table 1
The system suitability test results of the developed method for determination of PR, DT, CT, PS and their degradants.

Peak no.	Deg. medium	Compound	Retention time (min)	Capacity factor (k')	Selectivity, α	Resolution, R_s	Tailing factor
1	HCl/NaOH	PRD_1	1.8	0.64	2.25	4.47	1.10
2		PR	2.68	1.44	1.27	3.65	1.08
3	H_2O_2	DTD_1	3.11	1.83	1.60	3.10	1.14
4	H_2O_2	DTD_2	4.31	2.92	2.16	2.30	1.29
5	NaOH	DTD_3	8.06	6.32	1.13	3.05	1.25
6		PS	8.93	7.11	1.13	1.95	1.18
7	NaOH	DTD_4	9.94	8.02	1.15	9.65	1.20
8	H_2O_2	DTD_5	11.28	9.25	1.10	2.40	1.04
9		CT	12.26	10.14	1.06	4.66	1.04
10	H_2O_2	CTD_1	12.91	10.73	1.07	4.06	1.04
11		DT	13.72	11.47	1.34	4.48	0.95
12	HCl/H_2O_2	CTD_2	18.04	15.39	1.47	18.23	0.91
13	H_2O_2	CTD_3	25.96	22.62			1.01

Table 4
Characteristic parameters for the regression equations of the proposed HPLC method for determination of PR, DT, PS and CT.

Parameters	PR	DT	PS	CT
Calibration range ($\mu g\,mL^{-1}$)	2–115	0.5–50	1–50	0.5–50
Detection limit ($\mu g\,mL^{-1}$)	2.04×10^{-2}	3.85×10^{-2}	3.72×10^{-2}	3.70×10^{-2}
Quantitation limit ($\mu g\,mL^{-1}$)	6.81×10^{-2}	12.84×10^{-2}	12.40×10^{-2}	12.33×10^{-2}
Regression equation (Y)[a]				
Slope (b)	2.02×10^{4}	1.46×10^{4}	1.83×10^{4}	1.74×10^{4}
Standard deviation of the slope (S_b)	1.76×10^{2}	2.39×10^{2}	2.91×10^{2}	2.74×10^{2}
Relative standard deviation of the slope (%)	0.87	1.64	1.59	1.58
Confidence limit of the slope[b]	2.00×10^{4}–2.04×10^{4}	1.44×10^{4}–1.48×10^{4}	1.80×10^{4}–1.86×10^{4}	1.71×10^{4}–1.76×10^{4}
Intercept (a)	7.90×10^{3}	4.14×10^{3}	3.99×10^{3}	3.65×10^{3}
Standard deviation of the intercept (S_a)	1.24×10^{4}	5.94×10^{3}	8.10×10^{3}	7.53×10^{3}
Confidence limit of the intercept[b]	(-4.20×10^{3})–2.00×10^{4}	(-1.63×10^{3})–9.93×10^{3}	(-3.89×10^{3})–1.19×10^{4}	(-3.67×10^{3})–1.10×10^{4}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	6.85×10^{3}	3.92×10^{3}	5.13×10^{3}	4.90×10^{3}

[a] $Y = a + bC$, where C is the concentration of PR, DT, PS and CT in $\mu g\,mL^{-1}$ and Y is the peak area.
[b] 95% confidence limit.

results was performed using the P-value of the F-test. Three univariate analyses of variance for each concentration level were made. Since the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

3.5.3. Range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in the pharmaceutical product, to give accurate, precise and linear results. The calibration ranges of the proposed HPLC method are given in Table 4.

3.5.4. Detection and quantitation limits

According to the International Conference on Harmonization (ICH) recommendations [39], the approach based on the standard deviation (S.D.) of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Table 4.

Table 5
ANOVA (showing Lack of Fit calculation) for PR, DT, PS and CT.

Comp.	Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-ratio
PR	Total	4.33×10^{12}	21	2.06×10^{11}	2.80
	Regression	4.33×10^{12}	2	2.17×10^{12}	
	Residual	1.36×10^{9}	19	7.16×10^{7}	
	Replicate	2.35×10^{8}	7	3.35×10^{7}	
	Lack of Fit	1.13×10^{9}	12	9.38×10^{7}	
DT	Total	4.00×10^{11}	21	1.90×10^{10}	1.74
	Regression	4.00×10^{11}	2	2.00×10^{11}	
	Residual	3.06×10^{8}	19	1.61×10^{7}	
	Replicate	7.70×10^{7}	7	1.10×10^{7}	
	Lack of Fit	2.29×10^{8}	12	1.91×10^{7}	
PS	Total	7.33×10^{11}	21	3.49×10^{10}	1.63
	Regression	7.33×10^{11}	2	3.67×10^{11}	
	Residual	4.99×10^{8}	19	2.62×10^{7}	
	Replicate	1.32×10^{8}	7	1.88×10^{7}	
	Lack of Fit	3.67×10^{8}	12	3.06×10^{7}	
CT	Total	6.78×10^{11}	21	3.23×10^{10}	1.82
	Regression	6.78×10^{11}	2	3.39×10^{11}	
	Residual	4.97×10^{8}	19	2.61×10^{7}	
	Replicate	1.2×10^{8}	7	1.72×10^{7}	
	Lack of Fit	3.76×10^{8}	12	3.14×10^{7}	

The critical value of F-ratio is 3.57 at $\alpha = 0.05$.

3.5.5. Selectivity and specificity

Methods selectivity was achieved by preparing seven laboratory-prepared mixtures of the studied compounds at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed according to the previous procedures described under the proposed method. Satisfactory results were obtained (Table 6) indicating the high selectivity of the proposed method for simultaneous determination of PR and DT (mixture 1); and PR, CT and PS (mixture 2).

The specificity of a method is the extent to which it can be used for analysis of a particular analyte in a mixture or matrix without interference from other components.

In this assay, specificity was tested by analysis of solutions containing degradants produced in forced degradation studies and by determination peak homogeneity or purity.

The specificity was demonstrated by the HPLC chromatograms recorded for mixtures of PR, DT, CT, PS and their degradants dissolved in the mobile phase, indicating the method enabled highly selective analysis of the drugs. Well-resolved peaks for PR, DT, CT, PS and their degradants were observed (Figs. 2–4).

In addition, peak homogeneity or purity have been done by collecting the peak of interest and re-injected under high-resolution chromatographic conditions "alteration of the HPLC conditions by decreasing organic strength" [40]. Results from these procedures confirmed specificity of the method.

3.5.6. Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by HPLC method. For this reason, standard addition method was applied to two commercial pharmaceutical formulations containing these compounds. In application of standard addition method the mean percentage recoveries and their standard deviation for the proposed method for six replicates were calculated (Tables 7 and 8). According to the obtained results a good precision and accuracy was observed for this method. Consequently, the excipients in pharmaceutical formulations do not interfere in the analysis of these compounds in their pharmaceutical formulations.

3.5.7. Robustness

Variation of the pH of the mobile phase (A) by ±0.2 units, the ionic strength of sodium dihydrogen phosphate by ±5 mmol L^{-1}, heptane sulfonic acid sodium salt by ±0.5 mmol L^{-1}, and organic strength of the mobile phase by ±2% did not have significant effect on chromatographic resolution in HPLC method.

Table 6
Determination of PR, DT, CT, and PS in laboratory-prepared mixtures using the proposed HPLC method.

Sample no.	Concentration ($\mu g\,mL^{-1}$)				% recovery			
	PR	DT	PS	CT	PR	DT	PS	CT
1	2	20	40	40	101.2	99.6	100.2	99.6
2	110	25	1	10	100.2	100.4	99.1	100.6
3	60	5	4.5	1.5	99.6	99.1	99.6	98.9
4	30	30	30	30	100.4	99.6	100.5	100.4
5	100	50	50	50	99.9	100.1	100.1	100.3
6	4	10	8	6	100.8	100.6	100.9	100.9
7	80	1	16	20	99.7	101.2	100.6	99.5
Mean					100.26	100.09	100.14	100.03
S.D.					0.59	0.71	0.62	0.71

Table 7
Application of standard addition technique on Alercet Cold® capsule to the analysis of PR, CT, and PS using the proposed HPLC method.

Sample no.	Claimed conc. ($\mu g\,mL^{-1}$)			Added conc. ($\mu g\,mL^{-1}$)			% recovery of added conc.		
	PR	PS	CT	PR	PS	CT	PR	PS	CT
1	20	1.5	0.5	10	5	30	100.9	99.1	99.7
2	20	1.5	0.5	80	45	45	100.2	99.7	100.3
3	40	3	1	20	20	20	100.6	100.5	100.8
4	40	3	1	50	30	15	100.4	99.8	101.1
5	80	6	2	15	3	6	99.7	101.2	98.9
6	80	6	2	5	40	40	99.5	100.2	99.8
Mean							100.22	100.08	100.10
S.D.							0.53	0.73	0.80

Table 8
Application of standard addition technique on Dantrelax Compound® capsule to the analysis of PR, and DT using the proposed HPLC method.

Sample no.	Claimed conc. ($\mu g\,mL^{-1}$)		Added conc. ($\mu g\,mL^{-1}$)		% recovery of added conc.	
	PR	DT	PR	DT	PR	DT
1	12	1	88	2	100.2	98.8
2	12	1	8	44	100.8	99.8
3	48	2	20	40	99.3	100.3
4	48	2	40	20	99.8	100.5
5	84	7	7	7	99.4	101.1
6	84	7	26	33	99.7	100.4
Mean					99.87	100.15
S.D.					0.56	0.78

3.5.8. Analytical solution stability

To demonstrate the stability of standard working solutions and of capsules sample solutions during analysis, both solutions were analyzed over a period of 12 h while being stored at room temperature and for 24 h when refrigerated at 4 °C.

The results showed that the retention times and peak areas of the drugs remained almost unchanged and no significant degradation was observed during this period, suggesting that these solutions were stable for at least 12 h at room temperature or 24 h when refrigerated at 4 °C, which was sufficient for the whole analytical process.

4. Conclusion

Pharmaceutical product quality is of vital importance for patient safety. The presence of degradants may affect the efficacy and safety of pharmaceuticals. Potential degradants can change the chemical, pharmacological and toxicological properties of drugs and have a significant effect on product quality and safety. Drug stability is regarded as a secure way of ensuring delivery of therapeutic doses to patients.

In this work a simple, sensitive, accurate, linear, precise, reproducible, repeatable, specific, and robust stability-indicating HPLC method was established for determination of PR, DT, CT and PS in the presence of their degradants. The behavior of PR, DT, CT and PS under different stress conditions was studied. The method is sensitive enough for quantitative detection of the analytes in pharmaceutical preparations and can thus be used for routine analysis, quality control, and for checking quality during stability studies of pharmaceutical preparations containing these drugs.

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