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Research Article

Development and Validation of HPLC Methods for the Determination of Propranolol Hydrochloride and Hydrochlorothiazide Related Substances in Combination Tablets

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Abstract

A recent study shows that propranolol hydrochloride (PRO) related substances may also reduce the stability of tablets in storage. Therefore, it is necessary to control the level of PRO related substances in tablets. However, the analysis in U.S. pharmacopoeia could not detect PRO related substances. To overcome this, we developed a new method which can detect PRO, hydrochlorothiazide (HCT) and all of their impurities. In this study, validation studies were also performed, linear relationship with a good correlation coefficient (r^2)>0.990 was found of both PRO impurities and HCT impurities in the range of 0.12-0.60, and 0.15-0.75 µg/ml respectively. Acceptable intra- and inter- assay precisions were achieved. Accuracy and robustness were reported as percent recovery, and all the recoveries were at the range of 70-130%. After validation, the methods were successfully used in the routine quality control of the tablets.

Keywords: Propranolol hydrochloride; Hydrochlorothiazide; Related substances; HPLC; Pharmaceutical analysis

Introduction

Propranolol hydrochloride and hydrochlorothiazide tablets, U.S. pharmacopoeia (USP) for oral administration, combine two antihypertensive agents [1]. Propranolol hydrochloride, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride, is a nonselective beta-adrenergic blocking agent possessing with no other autonomic nervous system activity [2,3]. It specifically competes with beta-adrenergic receptor stimulating agents for available receptor sites [4]. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a "thiazide" class diuretic. It reduces blood volume by increasing the excretion of sodium, chloride and water [5,6]. The decrease in blood volume, however, causes counter-regulatory stimulation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system [7,8]. Based on the different pharmacological mechanisms of the above two drugs and the character of activating the RAAS of HCT, PRO and HCT combination tablets were developed to treat hypertension.

The tablets were included in the USP35/NF30, but only one impurity of HCT, Benzothiadiazine Related Compound A (Ben), was involved in the method. Recent studies showed that PRO related substances such as Impurity A (IA), Impurity B (IB) and Impurity C (IC), and HCT related substances such as Chlorothiazide (Chl) and 5-chlorohydrochlorothiazide (5-Cl) may also reduce the shelf life of PRO and HCT combination tablets [9-11]. However, the analytical method in U.S. pharmacopoeia could not detect the PRO related substances and the other two related substances of HCT. To overcome this, the method for the determination of main drugs and their related substances is extremely necessary. The goal of our study was to develop and validate accurate, selective and HPLC sensitive methods for the determination of both main drugs and all of the related substances known in the compounds. We used two systems to detect PRO related substances (system A) and HCT related substances (system B) respectively. The methods were validated in terms of system suitability, sensitivity, linearity, accuracy, precision and robustness.

Materials and Methods

Materials

Propranolol hydrochloride and hydrochlorothiazide were obtained from NIFDC (National Institutes for Food and Drug Control). IA (purity: 98.3%), IB (purity: 98.3%) and IC (purity: 99.95%) were provided by Laboratory of the Government Chemist (Germany). 5-Cl (purity: 98.0%) was purchased from Toronto Research Chemicals (Canada). Ben (purity>98.0%) and Chl were procured from Tokyo Chemical Industry (Japan). Acetonitrile and Methanol of HPLC grade were purchased from Fisher. Water was obtained from Wahaha (Wahaha, Hangzhou). All other chemicals were of analytically reagent grade.

Equipment

The HPLC methods were all developed and validated by using a Shimadzu HPLC system, consisting of DGU-20A degasser, LC-20AT VP solvent pump, CTO-20AC column oven and SIL-20A auto sampler. The system also included SPD-M20A diode array detector and a computer running software (LC solution) for data acquisition and processing. The chromatographic separation was performed using a Phenomenex C_{18} column (150 mm × 4.6 mm, 5 µm). In system A, mobile phase A was pumped in isocratic mode at a flow of 1.0 ml/min at 25°C; while in system B, mobile phase B was pumped in isocratic mode at a flow of 1.5 ml/min at 30°C. The analytical wavelength was set at 292 nm for system A and 270 nm for system B.

Method development

Mobile phase: Mobile phase A- Mix 1.6 g sodium dodecyl sulfate and 0.31 g tetrabutylammonium dihydrogen phosphate in a mixture of 1 ml sulfuric acid and 450 ml water and add 550 ml acetonitrile. Adjust to pH 3.30 by using dilute sodium hydroxide solution.

Buffer- Dissolve 6.8 g monobasic potassium phosphate in 1000 ml water in a 2000 ml volume flask. Add 3.4 ml phosphoric acid and a volume of tetrabutylammonium hydroxide solution equivalent to about 2.6 g tetrabutylammonium hydroxide, then dilute with water to volume, and mix. Adjust, if necessary, with phosphoric acid or

potassium hydroxide (10 M) to pH 2.5 ± 0.1, and pass through a filter having a 0.45 µm or finer porosity. Mobile phase B- Prepare a suitable mixture of buffer and methanol (850/150, v/v).

Stock solutions and system suitability solutions: Propranolol hydrochloride -- A stock solution of PRO was prepared in mobile phase A at a concentration of 1.0 mg/ml. Stock solutions were individually prepared for IA, IB, and IC by diluting an accurately weighted amount of each drug in mobile phase A to yield drug concentrations of 0.4 mg/ml. An appropriate volume of each stock solution was diluted with mobile phase A to yield a mixture of system suitability solution A that PRO was 200 µg/ml and IA, IB, IC was 0.4 µg/ml.

Hydrochlorothiazide- HCT stock solution was prepared in mobile phase B at a concentration of 0.5 mg/ml. Stock solutions were individually prepared for Ben, Chl, and 5-Cl by diluting an accurately weighted amount of each drug in mobile phase A to yield drug concentrations of 0.1 mg/ml. Appropriate dilutions of stock solutions of HCT and HCT impurities were made with mobile phase B to obtain System suitability solution B that HCT was 50 µg/ml and Ben, Chl, 5-Cl were 0.5 µg/ml.

Test solutions and reference solutions: Propranolol hydrochloride- Weigh and finely powder not fewer than 20 tablets. Transfer an accurately weighed portion of the powder equivalent to about 100 mg PRO to a 100 ml volumetric flask. Add mobile phase A, mix and sonicate for 5 minutes with occasional swirling, then dilute with mobile phase A to volume, and mix. Filter a portion through a 0.45 µm solvent resistant filter (Test solution A). Quantitatively dilute with mobile phase A to an approximate concentration of 0.4 µg/ml of PRO (Reference solution A).

Hydrochlorothiazide- Weigh and finely powder not fewer than 20 tablets. Transfer an accurately weighed portion of the powder equivalent to about 5 mg HCT to a 100 ml volumetric flask. Add 1 ml water, mix and allow standing for 1 minute with occasional swirling. Add 15 ml methanol, mix, and sonicate for 5 minutes with occasional swirling. If necessary, add ice to the bath to maintain the temperature at not more than 20°C. Add 75 ml buffer, mix, and sonicate for 5 minutes with occasional swirling, maintaining the temperature of the bath at not more than 20°C. Dilute with buffer to volume and mix. Filter a portion through a 0.45 µm solvent resistant filter (Test solution B). Quantitatively dilute with mobile phase B to an approximate concentration of 0.5 µg/ml of HCT (Reference solution B).

Method validation

System suitability testing: As an informative part of the HPLC method development, system suitability was checked to evaluate the chromatographic performance of HPLC instrumentation. Chromatographic parameters related to the method must be within the system suitability limits before sample analysis can commence. The injection repeatability, tailing factor (T), theoretical plate number (N) and resolution (R_s) for the principal peaks were evaluated by using suitability solution A and suitability solution B. The resolution factor (R_s) should not be less than 1.5, and six successive injections should provide a relative standard deviation of less than 2.0%.

Specificity: The specificity of the method was investigated by observing any interference encountered tablet excipients and whether the two conditions interfered with each other.

Degradation studies: Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the procedure. The degradation samples were prepared for two systems respectively. Intentional degradation was attempted by using acid, base, hydrogen peroxide, heat and light. The samples were analyzed against a control sample (no degradation treatment).

LOD and LOQ: The limit of detection (LOD) and limit of quantitation (LOQ) decide about the sensitivity of the method. LOD is the lowest concentration of the analyte detected by the method, whereas LOQ is the minimum quantifiable concentration. The signal-to-noise ratio of 3:1 and 10:1 were taken as LOD and LOQ respectively, which was calculated using Shimadzu Class VP software and further confirmed by taking dilutions from the secondary stock solution till the peak area obtained has 3 (for LOD) and 10 (for LOQ) fold then the standard deviation of six determinations.

Linearity: A linear relationship was established by plotting the peak area ratio against the drug concentrations. The concentration ranges were found to be linear in the range of 0.12-0.60 and 0.15-0.75 µg/ml for PRO impurities and HCT impurities respectively.

Accuracy: To prove the accuracy, 9 determinations over 3 concentration levels covering the specified range (Low, Med and High) were used. Accuracy was reported as percent recovery by the assay of known amount of analytes added to the sample, while limits for the accuracy were set at the range of 70-130%.

Precision: Intra- and inter- precision were assessed in the assay of six samples by two different analysts using different chromatographic systems in different days. The intra- and inter- precision acceptance criteria for the related substances method set in the validation were: for each set (analyst) of data percentage RSD ≤ 15% and for all data combined (intra- and inter- precision data) percentage RSD ≤ 20%.

Robustness: Robustness of the method was evaluated in the assay with the deliberate changes in the experimental parameters which include propranolol hydrochloride (mobile phase pH 3.30 ± 0.10, mobile phase flow rate 1.0 ± 0.1 ml/min, column temperature 25 ± 5°C and different batches column) and hydrochlorothiazide (mobile phase pH 2.50 ± 0.10, mobile phase flow rate 1.5 ± 0.1 ml/min, column temperature 30 ± 5°C and different batches column). Analyses were carried out that only one parameter was changed in the experiments at a time.

Results and Discussion

HPLC system suitability and sensitivity

The obtained values of system suitability are given in Tables 1 and 2. The retention times of PRO, IA, IB and IC were 2.5, 1, 2, and 3 min. PRO and impurities can be finely separated. The resolution factor (R_s) was less than 1.5, and six successive injections of the suitability solution A provided a relative standard deviation of less than 2.0%. Suitability solution B met the same requirement. These results assured the suitability of the proposed HPLC method for routine simultaneous analysis of PRO, HCT and all their impurities involved (Figure 1). LOQ values were found to be 2.0-60.0 ng/ml and LOD values were found to be 0.8-21.0 µg/ml for PRO, IA, IB, IC, HCT, Ben, Chl and 5-Cl respectively (Tables 1 and 2).

Assay specificity and degradation studies

No significant interfering peaks from pharmaceutical excipients were observed near the retention time of the analytes (PRO, HCT and all of their impurities). Under the PRO chromatographic conditions, HCT and HCT related substances caused no interference to PRO and its related impurities. Similarly, there were no PRO peaks at the retention times of HCT and its related substances under HCT chromatographic

Parameter	IA	PRO	IB	IC
Chromatography retention time (min)	2.763	4.961	26.405	31.534
Tailing factor (T)	1.455	0.891	1.006	0.996
Number of theoretical plates (N)	4153	6206	9372	12894
Resolution (R_s)	—	10.385	31.936	4.659
Successive injections RSD (%)	0.9	0.1	0.9	1.1
Linearity range (μg/mL)	0.12-0.61	0.12-0.59	0.12-0.59	0.12-0.59
Correlation coefficient (r^2)	0.998	0.998	0.996	0.998
LOQ (ng/mL)	10.7	2.0	72.0	36.0
LOD (ng/mL)	4.3	0.8	60.0	18.0

Table 1: Analytical and chromatographic parameters of the HPLC method of propranolol hydrochloride.

Parameter	Ben	Chl	HCT	5-Cl
Chromatography retention time (min)	4.806	8.467	9.285	25.212
Tailing factor (T)	1.125	1.057	1.039	1.036
Number of theoretical plates (N)	6415	8259	8252	10102
Resolution (R_s)	—	11.948	2.094	22.556
Successive injections RSD (%)	0.7	0.9	0.1	0.9
Linearity range (μg/mL)	0.15-0.77	0.15-0.75	0.15-0.74	0.15-0.85
Correlation coefficient (r^2)	1.000	1.000	1.000	0.999
LOQ (ng/mL)	10.2	40.5	10.0	60.0
LOD (ng/mL)	3.0	15.0	1.8	21.0

Table 2: Analytical and chromatographic parameters of the HPLC method of hydrochlorothiazide.

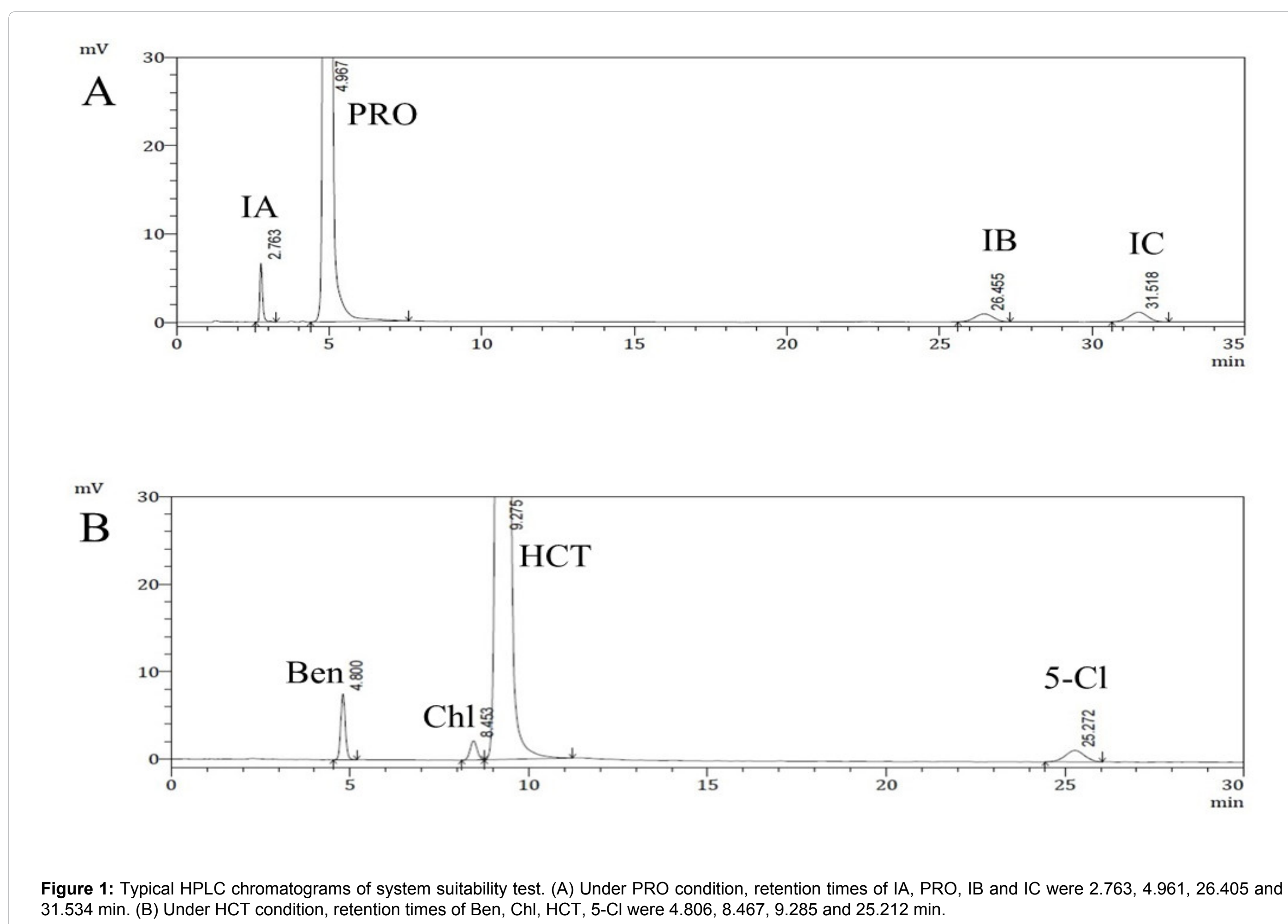

Figure 1: Typical HPLC chromatograms of system suitability test. (A) Under PRO condition, retention times of IA, PRO, IB and IC were 2.763, 4.961, 26.405 and 31.534 min. (B) Under HCT condition, retention times of Ben, Chl, HCT, 5-Cl were 4.806, 8.467, 9.285 and 25.212 min.

conditions (Figure 2). The percentage of PRO and HCT recovered is shown in Table 3. The main degradation product of PRO was IA (2.7 min) and main degradation product of HCT was Ben (5.3 min). The forced degradation studies indicated that IA and Ben should primarily be monitored in the research of drug stability. It also explained why the method of USP defined the detection of Ben, which was the only impurity involved.

Linearity

Calibration curves were constructed by plotting the peak area ratio of drugs against their corresponding concentrations respectively. The standard calibration curves of PRO and its related impurities were linear over the concentration range of 0.12-0.60 μg/ml with the correlation coefficients (r^2)>0.990, while HCT impurities standard calibration curves were linear over the concentration range of 0.15-0.75 μg/ml with the correlation coefficients (r^2)>0.990 (Tables 1 and 2).

Accuracy and precision

The mean recovery of PRO/HCT related substances were found 86.5-105.7%. The data obtained from accuracy studies was shown in Table 4. Observed recoveries of the tablet samples revealed that the two system methods assured the accuracy of the assay. Precision values were based on the calculation of percentage RSD, and the results for the related substances were summarized in Table 5. No impurities have been detected in PRO test solutions, while in HCT test solutions, single impurity (Ben) and total impurities percentage RSD were 1.5-6.5%. Thus, the results for both analysts were generally considered equivalent.

Robustness

Robustness of an analytical procedure was a measure of its capacity to remain unaffected by small variations in method parameters and provides an indication of its reliability during normal usage. Tables 6 and 7 showed the results of robustness, verifying that these minor changes do not greatly affect the assay of the drugs studied.

Application of the method to the analysis of tablets

The validated analytical method was successfully applied for the pharmaceutical studies of PRO and HCT impurities in their bulk forms. Tablets containing both drugs (40 or 80 mg of PRO and 25 mg of HCT) were subjected to the analysis by the proposed method. The aforementioned results indicated the suitability and accuracy of our method and its applicability for routine quality control of PRO and HCT combined tablets without interference from the excipients.

Conclusion

We have successfully developed and validated a sensitive and accurate HPLC method for the detection of PRO, HCT and all their impurities in tablets. Our method showed acceptable selectivity, accuracy, precision, robustness and linear concentration ranges. This enables the method to process a large number of samples in pharmaceutical quality control.

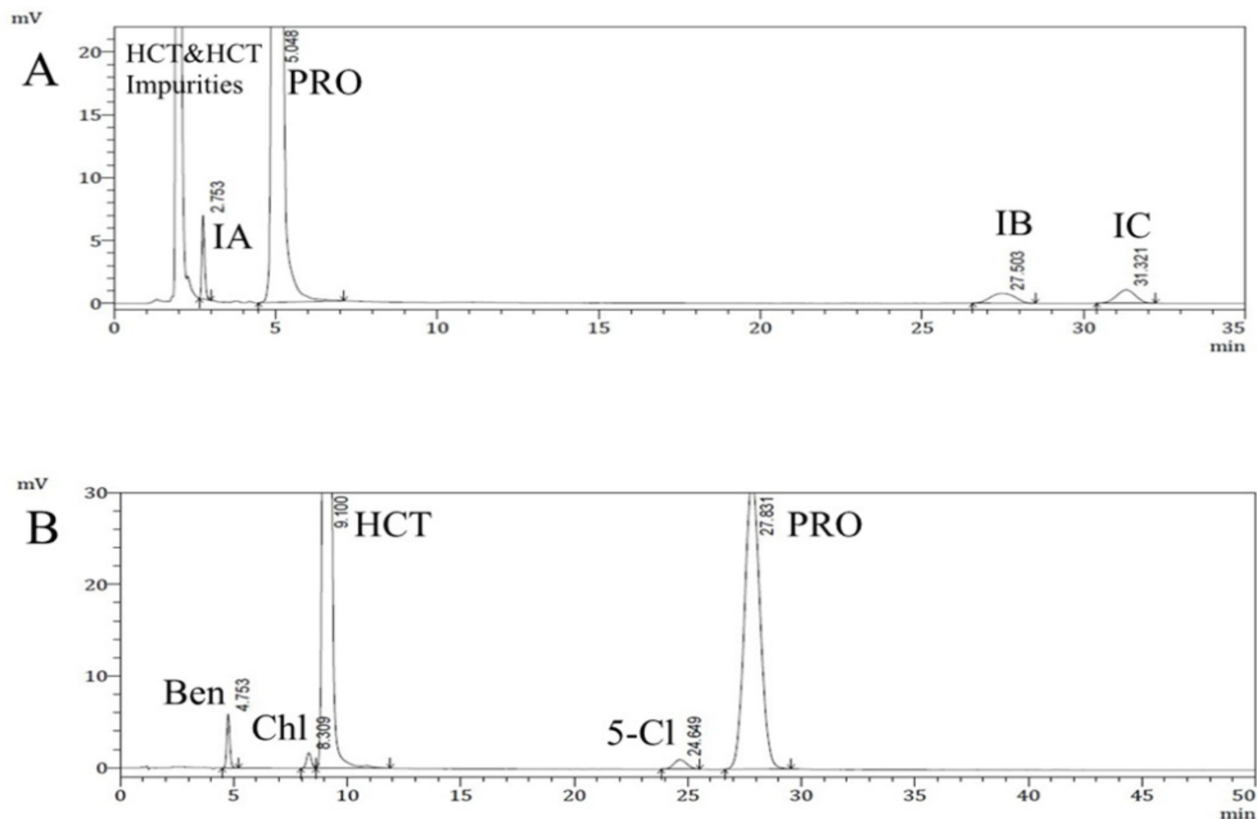

Figure 2: Representative HPLC chromatograms of specificity test. (A) In the condition of PRO, HCT and HCT impurities appeared before 2.5 min, whereas the first peak of PRO impurities, impurity A, appeared at 2.7 min. (B) PRO, the only peak among PRO and its related substances under the condition of HCT, appeared at 27.8 min, but still later than 5-Cl (24.6 min), which was the end of the peak of HCT related substances.

Condition of PRO	Time (h)	Recovery (%)	Ret[a] of degradation products
Acid 1.0 N HCl, 100°C	2	95.93	2.779, 5.950
Base 1.0 N NaOH, 100°C	0.5	91.57	6.806
Hydrogen peroxide 3%, 100°C	0.5	98.53	None detected
Heat dry, 100°C	2	100.55	None detected
Heat wet, 100°C	24	94.64	2.753
Light wet, 4500 lux	7	100.51	2.772
Condition of HCT	Time (h)	Recovery (%)	Ret[a] of degradation products
Acid 1.0 N HCl, 50°C	1	97.79	5.325
Base 1.0 N NaOH, 70°C	1.5	96.79	5.318
Hydrogen peroxide 3%, 100°C	1	96.53	5.301, 8.190
Heat dry, 100°C	24	99.55	4.915
Heat wet, 100°C	1	93.80	5.287
Light wet, 4500 lux	24	99.65	5.275

[a]Ret, relative retention time.

Table 3: Degradation of PRO and HCT.

	LOW			MED			HIGH		
	IA	**IB**	**IC**	**IA**	**IB**	**IC**	**IA**	**IB**	**IC**
Nominal Conc.	0.32	0.32	0.32	0.40	0.39	0.40	0.49	0.47	0.49
Recovery (%)	97.9	105.7	99.2	96.3	104.0	98.5	96.7	105.5	100.0
	LOW			**MED**			**HIGH**		
	Ben	**Chl**	**5-Cl**	**Ben**	**Chl**	**5-Cl**	**Ben**	**Chl**	**5-Cl**
Nominal Conc.	0.40	0.39	0.45	0.51	0.49	0.56	0.61	0.59	0.67
Recovery (%)	87.6	95.3	101.5	86.5	97.2	100.9	87.3	99.5	99.4

Table 4: Accuracy of the HPLC method for determination of IA, IB, IC, Ben, Chl and 5-Cl.

Sample	Analyst 1		Analyst 2	
	Single impurity (%)	**Total impurities (%)**	**Single impurity (%)**	**Total impurities (%)**
1	0.37	0.37	0.43	0.43
2	0.38	0.38	0.44	0.44
3	0.38	0.38	0.43	0.43
4	0.38	0.38	0.43	0.43
5	0.39	0.39	0.42	0.42
6	0.39	0.39	0.43	0.43
Mean (6)	0.38	0.38	0.43	0.43
RSD (%)	2.0	2.0	1.5	1.5
Mean (12)	0.41	0.41	—	—
RSD (%)	6.5	6.5	—	—

Table 5: Method precision of HCT related substances.

Parameter	Recovery (%)		
	IA	**IB**	**IC**
The recommended condition[a]	94.9	102.3	97.8
PH of the mobile phase			
3.20	103.4	112.1	107.4
3.40	104.6	112.3	106.7
Flow rate (mL/min)			
0.9	96.0	101.4	98.7
1.1	96.0	104.4	101.9
Column temperature (°C)			
20	116.2	115.5	113.0
30	114.5	118.0	113.5
Batches of column	103.5	116.4	110.3

[a]The recommended conditions were given in the Experimental section.

Table 6: Robustness of the HPLC method for determination of IA, IB and IC.

Parameter	Recovery (%)		
	Ben	**Chl**	**5-Cl**
The recommended condition[a]	98.6	102.6	105.4
PH of the mobile phase			
2.40	99.9	104.1	89.2
2.60	82.0	105.9	88.3
Flow rate (mL/min)			
1.4	107.2	104.3	104.1
1.6	94.1	103.4	103.6
Column temperature (°C)			
25	100.7	107.1	86.1
35	103.8	102.9	92.5
Batches of column	95.8	92.8	108.0

[a]The recommended conditions were given in the Experimental section.

Table 7: Robustness of the HPLC method for determination of Ben, Chl and 5-Cl.

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