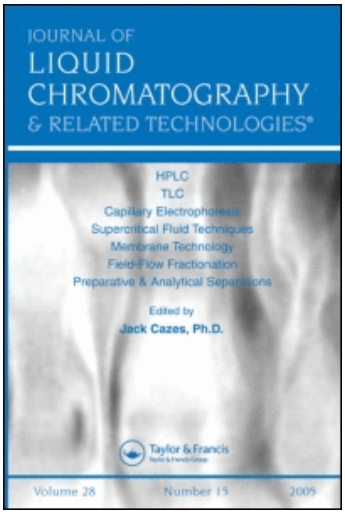

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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING LC METHOD FOR THE DETERMINATION OF DOMPERIDONE, SORBIC ACID, AND PROPYLPARABEN IN PHARMACEUTICAL FORMULATIONS

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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING LC METHOD FOR THE DETERMINATION OF DOMPERIDONE, SORBIC ACID, AND PROPYLPARABEN IN PHARMACEUTICAL FORMULATIONS

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□ *A simple, selective, and sensitive stability-indicating LC method has been developed and validated for the simultaneous determination of sorbic acid, propylparaben, and domperidone in pharmaceutical oral suspension formulations. The separations was achieved on a Lichrosorb C_8, 150 mm × 4.6 mm and 5 μm column with detection of 280 nm using an isocratic mobile phase mixture of phosphate buffer (0.05 M) and methanol (40:60 v/v) at flow rate of 1.0 mL/min. Under these conditions, separation of the three components was achieved in less than 10 min. The retention times for sordid acid, propylparaben, and domperidone were found to be 3.88, 6.12, and 8.53 min with good resolution of 7.90 and 6.11, respectively. The calibration curve for sorbic acid, propylparaben, and domperidone was linear in the range of 30–150, 5–30, and 36–180 μg/mL, respectively with $r = \geq 0.9998$ for each component. The proposed method was successfully employed for quantification of sorbic acid, propylparaben, and domperidone in pharmaceutical formulations.*

Keywords domperidone, LC, pharmaceutical preparation, propylparaben, sorbic acid, stability-indicating method

INTRODUCTION

Domperidone (DP) (4-(5-chloro-2-oxo-1-benzimidazolinyl)-1-[3-(2-oxobenzimidazolinyl) propyl]piperidine, $C_{22}H_{24}ClN_5O_2$, Figure 1) is an antidopaminergic drug used in tablet, oral suspension, and suppositories formulations. It stimulates gastro-intestinal motility and is used as an antiemetic for the short term treatment of nausea and vomiting of various aetiologies, including that associated with cancer therapy and with levodopa or bromocriptine therapy for parkinsonism.[1] Some organic acids and their esters are commonly used single preservatives, but more often

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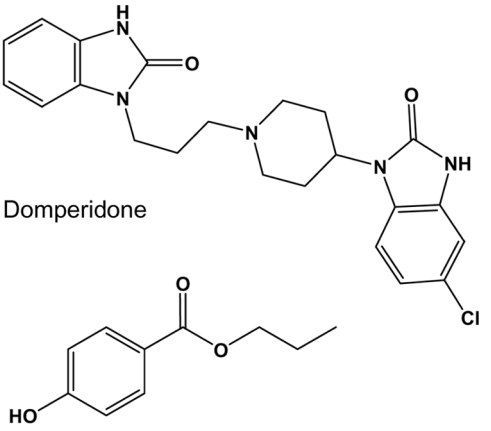
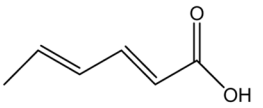


Domperidone

Propylparaben (Propyl 4-hydroxybenzoate)



Sorbic acid (2,4-hexadienoic acid)

FIGURE 1 Chemical structures of the separated compounds.

combinations of preservatives as antimicrobial agents in cosmetic, food, and pharmaceutical products[2] to prevent chemical alteration and degradation of the product formulation. Sorbic acid (SA) is generally effective to control mold, inhibits yeast growth, and effective against a wide range of bacterial attacks.[3] Propylparaben (PP) is the most commonly used preservative and has been used for many years. It had been found that the antimicrobial activities of the parabens seem to increase with increasing chain length. However, esters of longer alkyl chains are of limited applications due to their lower solubility in water.[4] The analysis of these preservatives in commercial pharmaceutical products is particularly important both for quality assurance and consumer protection.

Domperidone is also known as a dopamine D_2 receptor antagonist used as an antiemetic agent in human beings for the prevention of nausea and vomiting. It is widely used all over the world for its unique pharmaceutical activity. Therefore, the analysis of DP into oral suspensions in combination with preservatives is required and urgently needed. Some LC methods are available on the determination of DP, either alone or in combination with other drugs.[5–8] Several analytical procedures have been reported for the determination of SA and PP preservatives separately or in combination with other drugs by LC and other techniques.[9–21] These methods may not be

suitable for simultaneous determination of SA, PP, and DP together in one chromatographic run. However, after a thorough literature search, no LC method was found in oral suspension for the simultaneous determination of these compounds containing a combination of the three components together, SA, PP, and DP. Therefore, attempts were made in this study to develop a fast, sensitive, selective, and robust method for the simultaneous determination of SA, PP, and DP in oral suspension formulations. The present research describes the analysis of SA, PP, and DP in the pharmaceutical oral suspension formulations by liquid chromatography.

EXPERIMENTAL

Chemicals and Reagents

Methanol (HPLC-grade), domperidone (≥98% HPLC), sorbic acid (≥99.0%), propylparaben (≥99.0%), and potassium dihydrogen phosphate (≥99.0%) were purchased from Sigma-Aldrich (Gillingham, UK). Orthophosphoric acid (85%) was obtained from Merck Chemicals (Nottingham, UK). Purified water was prepared by Milli-Q system (Bedford, MA, USA).

Instrumentation and Conditions

Chromatographic separation was carried out on a Knauer HPLC system (Berlin, Germany) equipped with a model 1000 LC pump, model 3950 autosampler, model 2600 photodiode-array (PDA) detector, and a vacuum degasser was used. The data were acquired via Knauer ClarityChrom Workstation data acquisition software. The mobile phase consisted of a mixture of phosphate buffer (0.05 M) and methanol (40:60, v/v) was used. The flow rate was set to 1.0 mL/min. The injection volume was 20 μL and the detection wavelength was set at 280 nm. Reversed-phase LC analysis was performed isocratically at 30°C using a Lichrosorb C_8 (150 × 4.6 mm, 5 μm) column (Jones Chromatography, Hengoed, UK).

Standard Preparation

An accurately weighed amount (20 mg) of PP was placed in a 100 mL volumetric flask and dissolved in methanol to produce a standard solution (S1). An accurately weighed amount (12 mg) of DP and (10 mg) of SA were transferred into a 100 mL volumetric flask. A 50 mL of mobile phase was added and dissolved. A 10 mL aliquot of stock solution S1 was added, and volume was competed with mobile phase, yielding a final concentration of 0.12 mg of DP, 0.10 mg of SA, and 0.02 mg of PP/mL.

Sample Preparation

An accurately weighed amount (10.0 g) of sample suspension was transferred into a 100 mL volumetric flask. A 50 mL of mobile phase was added. This mixture was subject to sonication for 10 min for complete extraction of drug and preservatives and the solution was made up to the mark with mobile phase. The solution was centrifuged at 4000 rpm for 5 min; the clear supernatant portion was collected and filtered through a 0.22 μm membrane filter (Millipore, Watford, UK) and 20 μL of this solution was injected onto the HPLC system.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

For chromatographic separation of SA, PP, and DP a Lichrosorb C_8 stationary phase (4.6 mm, 5 μm) with varying column lengths from 250 to 150 mm was attempted. Different mobile phase compositions containing phosphate buffer (0.05 M) and methanol (50:50, 40:60, v/v) were tried. Although good separation was achieved with phosphate buffer and methanol in the ratio of 40:60 (v/v), DP peak symmetry was found to be greater than 2.0. The symmetry of the DP peak was improved by addition of 0.05% phosphoric acid in the mobile phase. The chromatographic separation with better peak shape was achieved using a mixture of aqueous 0.05% phosphoric acid and methanol in the ratio of 40:60 (v/v). The column, 250 mm × 4.6 mm, 5 μm showed higher elution time (4.46 for SA, 6.72 for PP and 9.12 min for DP) with resolutions of 8.15 for PP and 7.73 for DP. The shorter column length (150 mm × 4.6 mm, 5 μm) reduced the elution time (3.88 for SA, 6.12 for PP and 8.53 min for DP) with good resolution (7.90 and 6.11 USP), respectively. The overlaid photodiode-array (PDA) spectrum showed good response at 280 nm for all three components. Therefore, this wavelength was used for simultaneous determination of drug and both preservatives. In the optimized conditions, SA, PP, and DP were separated with a resolution of >6 and the retention times were found to be 3.88, 6.12, and 8.53 min, respectively. Chromatogram of system suitability, and suspension sample are shown in Figures 2 and 3.

Method Validation

The proposed method was validated with respect to linearity and range, specificity, precision, accuracy, limit of detection, limit of quantitation, robustness, and stability of analytical solutions following the International

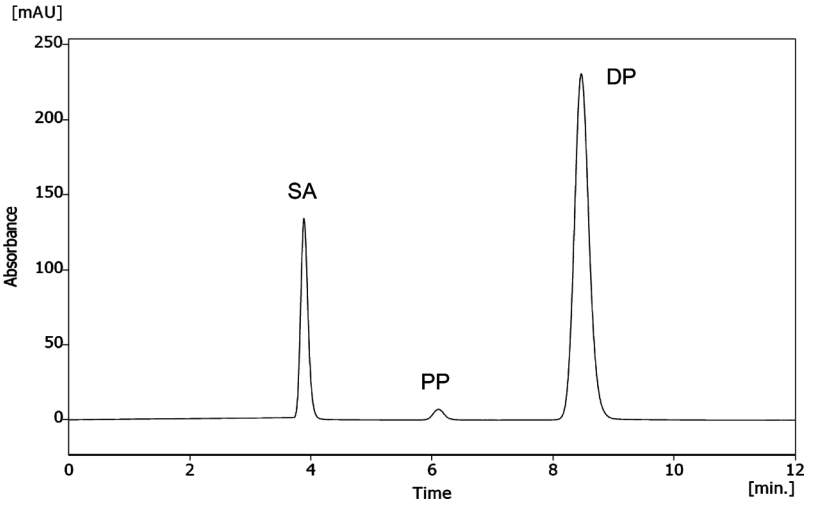


FIGURE 2 LC chromatogram obtained from standard during system suitability experiments.

Conference on Harmonization (ICH),[22] Shabir,[23] and United States Pharmacopeia (USP)[24] guidelines.

Linearity and Range

Linearity test solutions were prepared by diluting stock solutions of SA, PP, and DP (1 mg/mL) at six concentration levels from 30 to 150% levels of analytes concentration (30–150, 5–30, and 36–180 µg/mL, respectively).

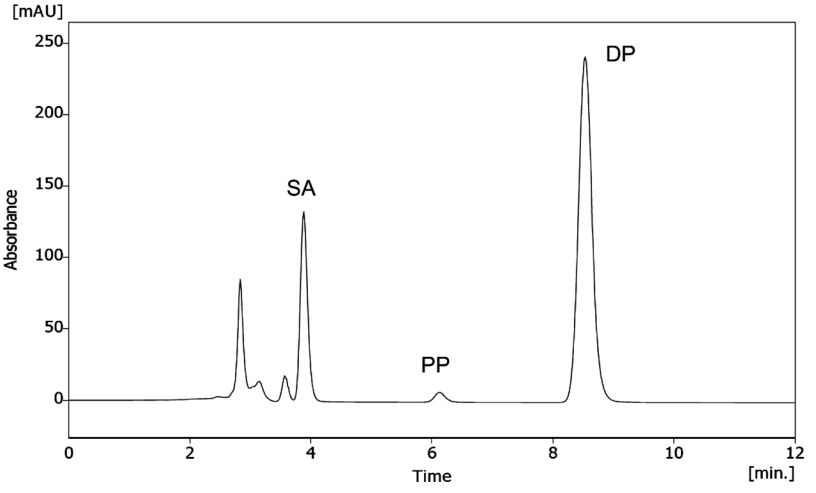


FIGURE 3 LC chromatogram obtained from pharmaceutical oral suspension sample.

TABLE 1 Linearity Results of the LC Method

Components	Concentration (μg/mL)	Equation for regression line	R^2
Sorbic acid	30–150	$y = 11.576x + 69.486$	0.9999
Propylparaben	5–30	$y = 12.721x - 14.737$	0.9998
Domperidone	36–180	$y = 31.667x - 403.5$	0.9999

The solutions were injected in triplicate and the following regression equations were found by plotting the peak area (y) versus the SA, PP, and DP concentration (x) expressed in mg/mL:

$$y_{SA} = 11.576x + 69.486 \quad (R^2 = 0.9999)$$

$$y_{PP} = 12.721x - 14.737 \quad (R^2 = 0.9998)$$

$$y_{DP} = 31.667x - 403.5 \quad (R^2 = 0.9999)$$

The determination coefficient (r^2) obtained (Table 1) for the regression line demonstrates the excellent relationship between peak area and the concentration of SA, PP, and DP.

Precision

The precision of the chromatographic method, reported as percent relative standard deviation (RSD), was estimated by measuring repeatability (intra-day assay precision) on ten replicate injections at 100% test concentration (0.10 mg of SA, 0.02 mg of PP and 0.12 mg of DP/mL). The RSD values for retention time (min) were 0.05, 0.09, and 0.07%; values for peak area were 0.26, 0.56, and 0.19%; and values for peak height were 0.87, 0.62, and 0.78% for SA, PP, and DP, respectively. The intermediate precision (inter-day variation) was studied using two LC systems over two consecutive days at three different concentration levels (80, 100, 120 μg/mL for SA, 15, 20, 25 for PP, 90, 120, 150 μg/mL for DP) that cover the assay range (80–120%). Three replicate injections were injected for each solution. The RSD values for both analysts were $\leq$0.67% (Table 2) and illustrated the good precision of this analytical method.

Accuracy

The accuracy was evaluated by the recovery of a known amount of SA, PP, and DP in synthetic mixture prepared by mixing SA, PP, and DP to placebo, to obtain concentration of 80–120% of normal analytical

TABLE 2 Results from Evaluation of the Intermediate Precision of the LC Method

	Day 1, Analyst 1		Day 2, Analyst 2	
Concentration (μg/mL)	Assay (%)*	%RSD	Assay (%)*	%RSD
Sorbic acid				
80	99.97	0.34	99.95	0.48
100	99.99	0.39	100.03	0.29
120	99.92	0.27	99.93	0.36
Propylparaben				
15	100.01	0.42	99.92	0.56
20	100.11	0.36	99.86	0.37
25	99.98	0.67	100.05	0.23
Domperidone				
90	99.71	0.18	100.02	0.12
120	99.58	0.14	100.13	0.16
150	100.07	0.10	100.23	0.10

*Mean of three replicate.

condition. Calculation of accuracy was carried out as the percentage of drug and preservatives recovered from the synthetic mixture of the drug and preservatives. Mean recovery (Table 3) for SA, PP, and DP from the formulation was between 99.51 and 101.5 ($n = 3$) indicating that the developed method was accurate for the determination of SA, PP, and DP in pharmaceutical formulation.

Specificity

Specificity is the ability of a method to measure analyte response in the presence of its potential impurities. Stress testing of the drug substance can

TABLE 3 Results from Evaluation of the Accuracy of the LC Method

Theoretical (% of target level)	Added amount (mg)	Amount founded (mg)	Mean recovery (%)*	RSD (%)
Sorbic acid				
80	2.06	2.10	101.94	0.22
100	4.12	4.14	100.48	0.16
120	6.00	5.98	99.66	0.27
Propylparaben				
80	2.02	2.03	100.49	0.35
100	4.08	4.06	99.51	0.28
120	6.05	6.03	99.67	0.49
Domperidone				
80	2.10	2.13	101.43	0.11
100	4.07	4.09	100.49	0.08
120	6.11	6.16	100.82	0.16

*Mean of three replicate.

TABLE 4 Results from Evaluation of the Forced Degradation Study of the LC Method

Stress conditions	Sample treatment	Retention time (min)			Assay (%)		
		SA	PP	DP	SA	PP	DP
Reference	Fresh solution	3.88	6.12	8.53	99.97	99.99	100.01
Acidic hydrolysis	0.5 M HCl for 4 h	3.84	6.12	8.52	99.74	99.85	99.98
Basic hydrolysis	0.5 M NaOH for 4 h	3.87	6.11	8.53	99.92	99.67	99.95
Oxidative	3.0% H_2O_2 for 5 h	3.86	6.09	8.51	99.87	99.67	99.92
Heat degradation	70°C for 1 h	3.82	6.10	8.51	99.83	99.57	99.82
Light degradation	UV Light for 24 h	3.85	6.11	8.52	99.97	99.91	99.68

help to identify likely degradation products, which can, in turn, help establish the degradation pathways and intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedure used. In the present study, injections of the blank were performed to demonstrate the absence of interference with the elution of the SA, PP, and DP. These results demonstrate that there was no interference from the other compounds and, therefore, confirm the specificity of the method.

Forced degradation studies were also performed to evaluate the specificity of drug product and each preservative under four stress conditions (heat, UV light, acid, base, oxidative). Solutions of drug and each preservative were exposed to 70°C for 4 hr, UV light using a Mineralight UVGL-58 light for 24 hr, acidic hydrolysis (0.5 M HCl) for 4 hr, basic hydrolysis (0.5 M NaOH) for 4 hr, and oxidative degradation (3.0% H_2O_2) for 5 hr. A summary data of the stress results is shown in Table 4, which showed no changes in retention times of drug and preservative components and no degradation peaks were observed. This was further confirmed by peak purity analysis on a DAD UV detector and, therefore, confirms the specificity of the method.

Limits of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by the calibration plot method.[22] A specific calibration plot was constructed using samples containing amounts of analytes in the range of LOD and LOQ. The values of LOD and LOQ were 0.14, 0.22, and 5 µg/mL and 25, 5, and 30 µg/mL for SA, PP, and DP, respectively, for 20 µL injection volume. LOD and LOQ were calculated by use of the equations:

$$\text{LOD} = Cd \times Syx/b$$

and

$$\text{LOQ} = Cq \times Syx/b$$

where *Cd* and *Cq* are the coefficients for LOD and LOQ, *Syx* is the residual variance of the regression, and *b* is the slope. Calculations were performed by using values of *Cd* and *Cq* of 3.3 and 10. Precision at the limits of quantitation and detection was checked by analysis of six test solutions prepared at three levels. The RSD values for peak area were less than 2% for LOQ and less than 5% for LOD solutions.

Solutions Stability

The solution stability of SA, PP, and DP in the assay method was investigated by leaving sample test solutions in tightly capped volumetric flasks at room temperature for 48 hr. the same sample solutions were assayed at 6 hr intervals up to the end of the study period against freshly prepared standard solutions. The RSD (%) of the assay of SA, PP and DP were calculated for the study period during solution stability experiments. The RSD values of the assay of SA, PP, and DP were less than 1.0%. No significant changes were observed during solution stability experiments. The results from these experiments confirm that sample solutions used during assay were stable up to the study period of 48 hr.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. To determine the robustness of the method the experimental conditions were deliberately altered and retention times (min), assay percent, peak tailing, number of theoretical plates, and resolutions were evaluated.

The mobile phase flow rate was 1.0 mL/min. This was changed by 0.1 units to 0.9 and 1.1 mL/min and the effect was studied. Similarly, the effect of column temperature was studied at 28°C and 32°C instead of 30°C. The effect of mobile phase composition was studied by use of phosphate buffer (0.05 M) and methanol 38:62 and 42:58 (v/v). The effect of detection wavelength was studied at 275 and 285 nm. For all changes in conditions, the sample was analyzed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay of SA, PP, and DP for all deliberate changes of conditions was within 98.09–99.90%. The summary of results is shown in Table 5.

System Suitability

The system suitability test was performed to confirm that the LC system to be used was suitable for the intended application. A standard solution

TABLE 5 Result from Evaluation of the Robustness Study of the LC Method

Condition	Retention time (min)	Assay (%)	USP peak tailing	USP resolution	Theoretical plates
Flow rate (±10% of the optimum flow)					
0.9 mL/min	3.9, 6.2, 8.6	99.6, 99.9, 99.8	1.1, 0.7, 1.0	7.9, 6.2	4699, 5182, 5673
1.1 mL/min	3.7, 6.0, 8.4	99.7, 99.8, 99.5	1.0, 0.8, 0.9	7.8, 6.0	4684, 5168, 5665
Mobile phase composition (±2% of optimum organic modifier concentration)					
58 mL	4.0, 6.4, 8.8	99.8, 99.2, 99.7	1.0, 0.8, 1.0	7.8, 6.3	4694, 5172, 5678
62 mL	3.3, 5.9, 8.2	99.5, 98.8, 99.9	1.1, 0.9, 1.0	7.9, 6.2	4714, 5245, 5732
Temperature (±2°C of optimum temperature)					
28°C	3.8, 6.1, 8.4	99.7, 99.3, 99.8	1.0, 0.8, 1.0	7.8, 6.5	4689, 5179, 5677
32°C	3.9, 6.4, 8.7	99.4, 99.0, 99.5	0.9, 0.7, 0.9	7.7, 6.2	4694, 5176, 5682
Wavelength (±5 nm of the optimum wavelength)					
275 nm	3.9, 6.3, 8.6	99.2, 99.6, 99.2	1.2, 0.9, 1.0	7.6, 6.3	4699, 5180, 5675
285 nm	3.7, 6.1, 8.4	99.8, 99.2, 99.9	1.0, 0.8, 1.0	7.8, 6.2	4689, 5178, 5652

$n = 3$ determinations, data for SA, PP, and DP, respectively.

containing of 0.10 mg of SA, 0.02 mg of PP, and 0.12 mg of DP/mL was injected six times. The parameters measured were peak area, retention time, capacity factor, theoretical plate, and tailing factor. The RSD values calculated for the peak area were 0.43, 0.52, and 0.37 and retention times were 0.09, 0.07, and 0.06 for SA, PP, and DP, respectively. The tailing factors were 1.28, 1.40, and 1.15 for SA, PP, and DP, respectively. Theoretical plates were 4798, 5262, and 5756 and the resolutions were 4.96, 7.91, and 6.11 for SA, PP, and DP, respectively. The system suitability experimental results showed that the parameters evaluated were within the acceptable range (RSD < 2.0%) indicating that the system was suitable for the analysis intended applications.

Assay Results

Results from analysis of SA, PP, and DP pharmaceutical oral suspension products in different batches ($n = 3$) ranged from 99.94 to 99.98%.

CONCLUSION

The newly developed LC method is specific, precise, accurate, and rapid for the simultaneous determination of sorbic acid, propylparaben, and domperidone from pharmaceutical oral suspension formulation. An excellent correlation existed between peak areas and concentration of both drug and preservatives. It is a stability indicating method and suitable for

quality control of pharmaceutical preparations containing sorbic acid, propylparaben, and domperidone either alone or in combination.

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