SIMULTANEOUS DETERMINATION OF LEVODROPROPIZINE, METHYLPARABEN, AND PROPYLPARABEN IN ORAL CO-FORMULATED SYRUP BY RP-HPLC METHOD

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ABSTRACT

A selective, rapid, isocratic RP-HPLC method has been developed and validated for the simultaneous determination of the cough suppressant drug levodropropizine and its two co-formulated preservatives methylparaben and propylparaben in Levopront® syrups. The chromatographic separation was achieved on Inertsil® C18, (250 mm x 4.6mm, 5µm) column using a mixture of (acetonitrile: 0.1 % triethylamine in water, pH 3.0) 50:50: (v/v), as a mobile phase with a flow rate of 1.0 ml/min, at 30° C column temperature and detector wavelength of 240 nm. All peaks were symmetrical and well resolved in a short run time. Method validation demonstrated to be selective, accurate and precise with good linearity over the concentration range of (7.5–60 µg/ml), (1.625–13 µg/ml), and (0.25–2 µg/ml) with limits of detection and quantification of 0.502 and 1.520 µg/ml, 0.071 and 0.215 µg/ml, and 0.040 and 0.122 µg/ml for Levodropropizine, Methylparaben, and Propylparaben, respectively. Robustness against small modifications of column temperature, flow rate and pH of the mobile phase was ascertained. The developed method was successfully applied for the separation and quantification of Levodropropizine in presence of its two co-formulated preservatives in drug substances and in Levopront® oral pharmaceutical formulation; therefore it's highly suitable for routine analysis in QC labs.

Keywords: Levodropropizine; Methylparaben; Propylparaben; RP-HPLC; Method Validation.

1. INTRODUCTION

Levodropropizine (LDP) is a cough suppressant reported to have a peripheral action in non-productive cough. Chemically, LDP is (2S)-3-(4-Phenylpiperazin-1-yl) propane-1, 2-diol and is the (-)-(S)-isomer of dropropizine, it claimed to produce fewer CNS effects and is used similarly by mouth. Methylparaben (MP), methyl 4-hydroxybenzoate and Propylparaben (PP), propyl 4-hydroxybenzoate are alkyl esters of p-hydroxybenzoic acid with antibacterial and antifungal properties. Parabens are widely used as antimicrobial preservatives in aqueous pharmaceutical products to prevent decomposition by microbial growth ⁽¹⁾. However these preservatives may be harmful to consumer due to their tendency to induce allergic contact. Hence the simultaneous determination of these preservatives in commercial pharmaceutical products is particularly important both for quality assurance and consumer safety. The chemical structures of LDP, MP, and PP are shown in Figure 1.

LDP is an official drug, it was assayed in British pharmacopeia by potentiometric titration ⁽²⁾. Literature surveys revealed several HPLC methods for the determination of Levodropropizine in pharmaceutical products using UV-VIS spectrophotometric detector ⁽³⁻⁶⁾ and in biological fluids; using HPLC with UV-VIS spectrophotometric detector ⁽⁷⁾, fluorescence detector ⁽⁸⁻¹⁸⁾, tandem mass spectrometric detector ⁽¹⁹⁻²²⁾, and GC-MS detector ⁽²³⁻²⁵⁾. HPLC and TLC methods were reported for the separation of related substance in LDP ⁽²⁶⁾. Fatma et al presented electrochemical voltammetric methods for the determination of LDP in pharmaceuticals ⁽²⁷⁾. To the best of our knowledge; in the literature there is no reported isocratic HPLC method was found for the simultaneous determination of LDP, MP, and PP, so, it felt essential to develop and validate a selective, fast, precise and accurate HPLC method for the simultaneous determination of LDP, MP, and PP in drug substance and in co-formulated oral pharmaceuticals.

EXPERIMENTAL

Material and Reagents

Levodropropizine - EP (potency: 99.58%), was supplied by Chemswiss AG, Switzerland. Methylparaben - BP (potency: 99.74%), was supplied by AWA company. Propylparaben - BP (potency: 99.39%), was supplied by AWA company. Levopront® 30 mg/5ml Syrup, Armstrong Laboratorios, Mexico. Acetonitrile, HPLC grade (Labscan, Ltd, Dublin, Ireland). Methanol, HPLC grade (Labscan, Ltd, Dublin, Ireland). Triethanolamine, HPLC grade Fisher Scientific, UK. o-Phosphoric acid (Sigma Aldrich, Germany). Glass-distilled water

Levodropropizine, LDP

(2S)-3-(4-Phenylpiperazin-1-yl) propane-1,2-diol

Methylparaben, MP

Methyl 4-hydroxybenzoate

Propylparaben, PP

Propyl 4-hydroxybenzoate

Figure 1 Chemical structure and IUPAC names of LDP, MP, and PP

Instrumentation

An Agilent technologies LC system 1200 series consists of quaternary pump equipped with DAD detector, auto injector and degasser. A thermostatic reversed phase Inertsil® C18 column compartment (250mm x 4.6mm, 5µm) was used for separation of all the compounds. The chromatographic data were recorded using Dell computer system with LC solution data acquiring software (Chemstation 32, USA)

Chromatographic Conditions

All the samples were analyzed by HPLC on Inertsil® C18 column using (acetonitrile: 0.1 % triethylamine in water, pH 3.0) 50:50: (v/v) as a mobile phase, in an isocratic elution at a flow rate 1 ml/min. at 30° C column temperature. The detection was carried out by DAD at 240 nm. The mobile phase was filtered through a 0.45 µm nylon membrane filter.

Preparation of Standard Stock and Sample Preparation

Stock standard solution

An accurately weighed 300 mg LDP, 65 mg MP, and 10 mg PP working standards were transferred into 100-ml volumetric flask, dissolved in 60 ml methanol and volume completed with the same solvent.

Standard Preparation (For linearity)

Aliquots (0.25, 0.5, 0.75, 1, 1.25, 1.5, and 2 ml) of stock standard solution were accurately transferred into a series of 100-ml volumetric flasks. The volume was completed to the mark with mobile phase.

Standard Preparation (For LOD & LOQ)

An accurately weighed 10 mg LDP, 10 mg MP, and 10 mg PP working standards were separately transferred into a series of 10-ml volumetric flasks, dissolved in 60 ml methanol and the volume completed with the same solvent.

For LOD: Aliquots (5, 0.71, and 0.4 ml) of the previous LDP, MP, and PP solutions, respectively, were accurately transferred into a 100-ml volumetric flask and the volume was completed to the mark with methanol, 1 ml of this solution was accurately transferred into a 100-ml volumetric flask and the volume was completed to the mark with mobile phase.

For LOQ: Aliquots (15.2, 2.2, and 1.2 ml) of the previous LDP, MP, and PP solutions, respectively, were accurately transferred into a 100-ml volumetric flask and the volume was completed to the mark with methanol, 1 ml of this solution was accurately transferred into a 100-ml volumetric flask and the volume was completed to the mark with mobile phase.

Standard Preparation (For assay)

1.0 ml of stock standard solution was transferred into 100-ml volumetric flask and diluted with mobile phase and mixed well.

Sample Preparation

About 5.0ml of Levopront® syrup was accurately transferred into 100-ml volumetric flask & dissolved in 60 ml methanol, sonicated for 20 min, allowed to cool and then filtered through 0.45 µm membrane filter. 1 ml was accurately transferred into 10-ml volumetric flask and volume completed with mobile phase.

Method validation

After satisfactory development of the method, it was subjected to method validation as per ICH guidelines⁽²⁸⁾. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics system suitability, specificity, accuracy, precision, linearity, robustness, LOD and LOQ.

RESULTS AND DISCUSSION

In the literature there is no reported isocratic HPLC method for the simultaneous determination of levodropropizine, methylparaben, and propylparaben, so, the primary objective of this study was to develop an isocratic, accurate, simple, selective, reproducible, systematic and reliable HPLC method free of placebo interferences to separate and quantify levodropropizine and its co-formulated preservatives; methylparaben and propylparaben in a short run time and can be used for quality control laboratories.

Optimization of the chromatographic conditions

The main goal of this study is to establish a systematic, robust and reliable isocratic HPLC method to resolute and assay levodropropizine, methylparaben, and propylparaben in oral pharmaceutical solution; Levopront® syrup, so, detailed experimental design was constructed by systematic scouting of HPLC method components including mobile phase and pH conditions, Table (1). The method conditions were evaluated for peaks symmetry, peaks fronting, peaks tailing and the resolution. The final method conditions were selected.

Table 1 Scouting of parameters of HPLC

Parameters	Description of parameters
Mobile phase	Methanol: Water Methanol: Water: TEA 0.1% (pH 3.0) ACN: Water ACN: Water: TEA 0.1% (pH 3.0)
pH (water), adjusted with o-phosphoric acid	7.0 5.0 3.0

Method development using different mobile phases

All the trials are shown in Table 2.

Table 2 Observations and remarks of method development using different mobile phases.

Conditions	Observation	Remarks
Methanol: Water (50:50)	Good symmetrical sharp peaks of parabens while LDP peak was found to be asymmetrical.	Not Satisfactory
Methanol: Water: TEA (50:50:0.1), pH 3.0	Parabens and LDP peaks were found to be asymmetrical.	Not Satisfactory
ACN: Water (50:50)	Good symmetrical sharp peaks of parabens while LDP Peak was found to be asymmetrical.	Not Satisfactory
ACN: Water: TEA (50:50:0.1, pH 3.0)	Good symmetrical sharp peaks with accepted tailing factors and resolutions.	Satisfactory

Method development using different pH conditions

All the trials are shown in Table 3.

Table 3 Observations and remarks of method development in various pH conditions

Conditions	Observation	Remarks
ACN: Water: TEA (50:50:0.1, pH 7.0)	Good symmetrical sharp peaks of parabens while LDP Peak was found to be asymmetrical.	Not Satisfactory
ACN: Water: TEA (50:50:0.1, pH 5.0)	Parabens and LDP peaks were found to be asymmetrical.	Not Satisfactory
ACN: Water: TEA (50:50:0.1, pH 3.0)	Good symmetrical sharp peaks with accepted tailing factors and resolutions.	Satisfactory.

Final chromatographic conditions:

- **Column:** Inertsil®, ODS-3, C₁₈ column (250mm x 4.6 mm, 5µm)
- **Mobile phase:** (Acetonitrile: water: triethylamine, adjust pH to 3.0 with o-phosphoric acid) (50:50:0.1).
- **Temperature:** 30° C.
- **Flow rate:** 1.0 mL/ min.
- **Detector wavelength:** 240 nm.
- **Injection volume:** 20 µl.

Typical chromatogram is shown in Figure 2.

Figure 2 A typical Chromatogram of a mixture of levodropropizine, methylparaben, and propylparaben

System suitability

The system suitability parameters (Table 4) were evaluated by making the injection of the assay standard. The system was deemed to be suitable as the tailing factors for all the peaks were between 0.7 to 1.3 and the resolution between LDP, MP, and PP > 2.5

Table 4 The results for the system suitability of the assay standard

Component Reference value	Tailing factor 0.7-1.3	USP plate count >1500	Resolution > 2.5	Selectivity >1
Levodropropizine	0.81	1836	-	-
Methylparaben	0.80	4177	7.50	1.76
Propylparaben	0.80	4724	4.60	1.32

Method Validation

After satisfactory development of the method, it was subjected to method validation as per ICH guidelines⁽²⁸⁾.

Linearity

The linearity of the proposed method was constructed by triplicate analysis of different concentrations of LDP, MP, and PP standard solutions. The data was subjected to statistical analysis using linear regression model and the data were presented in Table 5. Linearity plots were shown in Figure 3.

Figure 3 Linearity of the peak area at 240 nm to the corresponding concentration of levodropropizine (7.5-60 µg/ml), methylparaben (1.625-13 µg/ml), and propylparaben (0.25-2 µg/ml).

Accuracy

Accuracy was assessed by analyzing five different concentrations within the linearity range. Three replicates of each concentration were analyzed and % recovery was found to be between 98.0 % and 102.0%. The validity of the proposed method was further assessed by applying the standard addition technique. The results are tabulated in Table 5.

Precision

The precision of the test method was evaluated by analyzing three different concentrations of LDP, MP, and PP, three replicate of each concentration were analyzed within a day to determine intra-day precision and over three days to determine the inter-day precision. The RSD was found to be below 2.0% which indicates the precision of the method for the quantification of LDP, MP, and PP. The results are shown in Table 5.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and the limit of quantification were established based on residual standard deviation of the response and the slope. The LOD was found to be 0.502µg/ml, 0.071µg/ml and 0.040µg/ml and LOQ was found to be 1.520µg/ml, 0.215µg/ml and 0.122µg/ml for LDP, MP, and PP, respectively.

Robustness

The robustness was investigated by varying the conditions with respect to the flow rate, column temperature and pH of the mobile phase. The study was conducted at three different flow rates (0.95ml/min - 1.00ml/min - 1.05ml/min), at three different column temperatures (28°C-30°C - 32°C) and at three different pH (2.5-3.0-3.5) to study the effect of these changes on the different chromatographic parameters. Negligible difference was found in system suitability parameters for LDP, MP, and PP such as USP plate count, resolution and the tailing factor (Table 6), so the method found to be robust.

Table 5 Validation parameters of the proposed method.

Parameter	Results		
	LDP	MP	PP
Slope	61.121	51.383	57.719
Intercept	27.529	7.336	2.154
Correlation coefficient	0.9998	0.9998	0.9998
Concentration range (µg/ml)	7.5 - 60	1.625 - 13	0.25 – 2
L.O.D	0.502	0.071	0.040
L.O.Q	1.520	0.215	0.122
Accuracy* Mean ± RSD			
Drug Substance	99.17 ± 0.526	99.46 ± 1.252	99.19 ± 0.922
Drug Product	105.26 ± 1.920	117.67 ± 0.505	99.36 ± 1.939
Standard Added	99.60±1.851	100.24 ± 0.571	99.84 ± 0.335
Precision** ± RSD			
Repeatability	0.384	0.796	1.260
Intermediate precision	0.759	1.142	1.606

*n=5 **n=9

Table 6 Robustness for the proposed HPLC method

Parameters	Flow rate (ml/min)			Column temperature (°C)			pH		
	0.95	1.00	1.05	28	30	32	2.5	3.0	3.5
Levodropropizine									
USP plate count	1830	1836	1832	1822	1836	1835	1831	1836	1835
Tailing factor	0.78	0.81	0.82	0.77	0.81	0.78	0.75	0.81	0.79
Methylparaben									
USP plate count	4168	4177	4180	4176	4177	4178	4169	4177	4172
Resolution	7.4	7.5	7.5	7.4	7.5	7.5	7.3	7.5	7.4
Tailing factor	0.79	0.80	0.81	0.78	0.80	0.81	0.78	0.80	0.78
Propylparaben									
USP plate count	4719	4724	4722	4718	4724	4723	4722	4724	4720
Resolution	4.5	4.6	4.7	4.5	4.6	4.7	4.5	4.6	4.5
Tailing factor	0.78	0.80	0.80	0.79	0.80	0.80	0.77	0.80	0.77

* Average of three determinations

The results obtained by applying the proposed method were statistically compared to those obtained by the reported methods^(2, 29); the values of the calculated t and F were less than the tabulated ones which revealed that there is no significant difference between them, Table 7

CONCLUSION

An isocratic, selective, rapid and validated HPLC method is described for the determination of levodropropizine in dug substances, and Levopront® syrup. Furthermore, two preservatives associated with drug formulation,

namely, methylparaben and propylparaben were successfully separated and quantified by this method without any interference from the blank, placebo, and any other additives. The proposed method was started with clear goals and the experimental design describes the scouting of the key HPLC method components. Their relationships are studied and the preliminary optimized conditions are obtained. All the validation parameters were found within accepted criteria. This developed method can be applied successfully to the quality control of the commercials and other routine analysis.

Table 7 Statistical comparison of the results obtained by the proposed HPLC method and the reported methods for determination of LDP, MP, and PP

Item	HPLC method			reported methods ^{*, (2, 29)}		
	LDP	MP	PP	LDP	MP	PP
Mean	99.17	99.46	99.19	99.09	99.22	100.19
S.D.	0.521	1.245	0.915	1.024	1.129	1.225
R.S.D. %	0.526	1.252	0.922	1.033	0.571	0.335
SE	0.233	0.557	0.409	0.458	0.505	0.548
Variance	0.271	1.566939	0.850	1.049	1.276	1.501
N	5	5	5	5	5	5
t test* (2.31)	0.156	0.317	1.471	-	-	-
F test* (6.39)	3.863	0.814	1.766	-	-	-

*The values between parenthesis are the theoretical values of t and F at (p = 0.05).

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