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Validation of liquid chromatographic method for analysis of lidocaine hydrochloride, dexamethasone acetate, calcium dobesilate, buthylhydroxyanisol and degradation product hydroquinone in suppositories and ointment

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Abstract

In this paper, there was developed a sensitive, precise and accurate reversed-phase liquid chromatographic (RP-HPLC) method and validated for simultaneous determination of lidocaine hydrochloride, dexamethasone acetate (DA) and calcium dobesilate (CD) in suppositories and ointment. Also there was achieved a parallel analysis of buthylhydroxyanisol, as a preservative, and hydroquinone, as a degradation product of calcium dobesilate, present in these dosage forms. The relative standard deviation (RSD) values for all five compounds indicated a good precision and accuracy of the RP-HPLC method. Method is selective, sensitive and reproducible with good recovery values and can be applied in simultaneous determination of all mentioned compounds.

Keywords: Liquid chromatography; Validation; Lidocaine hydrochloride; Dexamethasone acetate; Calcium dobesilate; Buthylhydroxyanisol; Hydroquinone

1. Introduction

Anti-haemorrhoidal (*Antihaemorrhoidalia*) medicines are used in symptomatic therapy, only partially as causal therapy of anorectic region disease. They are used locally, mostly as a combination of active compounds. Dosage forms that used are suppositories and ointments. Usual compounds used in this therapy are anaesthetics, anti-inflammatory drugs, antibiotics, vasoconstrictors, haemostatics, anticoagulants and antihistaminics [1].

Lidocaine hydrochloride (LH), as a local anaesthetic drug, reversibly inhibits nerve impulse transmission. It binds to the receptors in sodium channels and decreases their activity functioning as a cell membrane stabilizer. It has a good superficial activity, penetrates in depth through the mucous membranes and reduces the sensation of pain.

Dexamethasone acetate (DA) inhibits the inflammatory process, through the anti-inflammatory function, in the early stage. The function of glucocorticoids is not specific cause of decreased inflammatory reaction effects no matter what the cause of the reaction was [1].

Calcium dobesilate (CD) as a cyclohexadienolic bisulphate derivative, decreases the micro vascular permeability by inhibiting the histamine and bradiquinine concentration. In that way it reduces edema, inflammation and bleeding from hemorrhoids. It has a protective effect on blood vessels. Recent studies showed CD as an anti-oxidant improving endothelial function [2,3].

Considering the literature, the analytical methods for simultaneous qualitative and quantitative analysis of dexamethasone acetate, calcium dobesilate, lidocaine hydrochloride, butylated hydroxyanisole, and potential degradation product of calcium dobesilate-hydroquinone, in dosage forms were not described.

LH was investigated in rectal medical gel [4] and human plasma using high-performance liquid chromatographic

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(HPLC) [5,6] and LC–MS [7]. DA was determined by HPLC in cream [8], injection solutions [10] and equine serum [9]. DA was also determined in tablets using chemometrics-assisted spectrophotometry [11]. CA was directly determined in plasma, using a flow-injection biamperometric method [12] and a HPLC method after ion-pair extraction [13]. For the determination of butylated hydroxyanisole (BHA) in cosmetics [14] a GC–MS method was applied and a HPLC method was described for the determination of BHA in biological fluids [15]. No published study is available on the simultaneous HPLC separation and determination of all four compounds and potential degradation product hydroquinone (HQ) from suppositories and medical skin ointment. There is no scientific data about isocratic and gradient liquid chromatographic methods in parallel analysis of LH, DA and CD, as active substances.

Consequently the aim of this study was to develop the chromatographic method for the evaluation of mentioned five compounds in suppositories and ointment as dosage forms.

In this paper there was presented a novel reversed-phase high-performance liquid chromatographic method (RP-HPLC). Also, it was validated for the determination of five compounds in suppositories and ointment. The method describes the qualitative and quantitative analysis of the active components LH, DA and CD. The method involves the parallel analysis of BHA as a preservative and hydroquinone as a degradation product of CD. An isocratic RP-HPLC was developed for the simultaneously determination of all mentioned compounds in suppositories and ointment.

The method was validated by using the ICH guideline [16]. The selectivity, limits of detection and quantification, linearity, precision and accuracy were determined. Determination was carried out using Modolex suppositories and ointment. The RP-HPLC method that was presented has been proved successfully in separation and simultaneous determination of all five compounds.

2. Experimental

2.1. Apparatus and reagents

The analysis were carried out using a liquid chromatograph composed of a LC 1120 GBC (GBC Scientific Equipment, Dandenong, VIC Australia) chromatographic system equiped with GBC LC 1120 HPLC pump, GBC LC 1210 UV–vis detector and Rheodyne model 7125 (20 μL) sample loop injector. Chromatograms and peak areas were recorded and integrated using a WinChrom Chromatography Data System (GBC Scientific Equipment, Castle Hill). The column used was YMC Pack ODS AQ column (5 μm particle size, 250 mm × 4.6 mm) (Yamamura Chemicals, Kyoto, Japan). Water for chromatography was obtained using System Simplicity 185 (Millipore, Billerica MA, USA) purification system. The mobile phase was prepared daily, degassed and vacuum filtered prior to use through a Whatman 0.45 μm (47 mm diameter) nylon membrane filter (Whatman International, Maidstone, UK). Anotop 25, 0.2 μm o.d. syringe filters (Merck, Darmstadt, Germany) were used to filter the samples.

LH, DA, CD, BHA and HQ, standard substances, were purchased from Lek (Ljubljana, Slovenia). Modolex Plus ointment and Modolex Plus suppositories were originally formulated by Lek (Ljubljana, Slovenia). BHA content was declared as maximum allowed concentration in suppositories (0.45 mg per suppository) and ointment (0.20 mg g^{-1}). Maximum allowed content of hydroquinone was 0.10% in suppositories and ointment.

HPLC gradient grade acetonitrile and 85% orthophosphoric acid were obtained from Merck.

2.2. Chromatographic conditions

The mobile phase consisted of acetonitrile:water (50:50, v/v) and the pH was adjusted to 2.5 with 85% ortophosphoric acid. The mobile phase was degassed and vacuum filtered. The column was equilibrated before each run at a flow rate of 1.0 mL min^{-1} and the temperature was thermostated at 20 °C until a stable base line was achieved. Detection was done at two wavelenghts, channel A at 240 nm and channel B at 290 nm. The sensitivity was adjusted to 0.2 AU for channel A and 0.5 AU for channel B in order to screen the active substances. For screening of the BHA and hydroquinone, the sensitivity of channel B was switched to 0.1 AU.

2.3. Solutions

About 32.0 mg of LH was dissolved into the mobile phase and further diluted to 20.0 mL. Concentration of LH in working solution was 1.6 mg mL^{-1}.

A stock solution of DA was prepared in concentration 0.20 mg mL^{-1} by dissolving 10.0 mg of DA in 50.0 mL of mobile phase. Working solution of DA was prepared by diluting 1.0 mL of stock solution to 20.0 mL with mobile phase. The concentration of working solution was 10.0 μg mL^{-1} of dexamethasone acetate.

CD working solution was prepared by dissolving 80.0 mg of standard substance in 20.0 mL of mobile phase to obtain the concentration of 4.00 mg mL^{-1}.

Stock solution of BHA was prepared by dissolving 10.0 mg of standard substance in 10.0 mL. The concentration of this solution was 1.00 mg mL^{-1}. Working solution was obtained by diluting 1.0 of stock solution to 100.0 mL with mobile phase. The concentration of BHA in this solution was 0.01 mg mL^{-1}.

Hydroquinone (HQ) stock solution was prepared by dissolving 10.0 mg of standard substance in 25.0 mL of mobile phase (0.40 mg mL^{-1}). Working solution was obtained by diluting 1.0 mL stock solution in 20.0 mL mobile phase (0.02 mg mL^{-1} of hydroquinone).

System suitability solution was prepared by diluting 1.00 mL of working solution of LH, 1.00 mL of dexa-

methasone acetate working solution, 2.50 mL of CD working solution, and 2.0 mL of BHA working solution and 1 mL of hydroquinone working solution to 10.0 mL with mobile phase. The concentrations of LH, DA, CD, BHA and HQ were 0.16 mg mL^{-1}, 1.00 μg mL^{-1}, 1.00 mg mL^{-1}, 2.00 μg mL^{-1} and 2.00 μg mL^{-1}, respectively.

2.3.1. Preparation of standard curve of LH

0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.2, and 1.3 mL of working solution of LH-hydrochloride monohydrate were transferred into volumetric flasks of 10.0 mL and diluted to volume with mobile phase to obtain the concentrations from 0.064 to 0.208 mg mL^{-1}.

2.3.2. Preparation of standard curve of DA

0.3, 0.4, 0.5, 0.6, 1.0, 2.0, 2.4, and 3.0 mL of working solution of DA were transferred to volumetric flasks of 10.0 mL and diluted to volume with mobile phase to make concentrations from 0.30 to 3.0 μg mL^{-1}.

2.3.3. Preparation of standard curve of CD

0.2, 0.4, 0.64, 0.8, 0.96, 1.0, 1.25, and 1.5 mL of working solution of CD were transferred into volumetric flasks of 10.0 mL and diluted to volume with mobile phase to make concentrations from 0.08 to 0.60 mg mL^{-1}.

2.3.4. Preparation of standard curve of BHA

0.8, 0.9, 3.2, 3.6, 4.0, 4.5, 4.8 and 5.4 mL of working solution of BHA were transferred into volumetric flasks of 10.0 mL and diluted to volume with mobile phase to make concentrations from 0.8 to 5.40 μg mL^{-1}.

2.3.5. Preparation of standard curve of HQ

0.16, 0.64, 0.80, 0.96, 1.5, 2.0, 2.5, and 3.0 mL of working solution of hydroquinone were transferred into volumetric flasks of 10.0 mL and diluted to volume with mobile phase to make concentrations from 0. 32 to 6.00 μg mL^{-1}.

All solutions were prepared in dark glass volumetric flasks that cause of the light sensitivity of the substances.

2.3.6. Suppositories sample preparation

Each of ten suppositories was transferred to a 25 mL volumetric flask and dissolved with about 15 mL of mobile phase using an ultraconic bath for 15 min, diluted to volume with mobile phase and filtered. 0.50 mL of each solutions was transferred to the 10 mL volumetric flask and diluted to volume with mobile phase. The expected concentrations of active compounds LH-hydrochloride, dexamethasone acetate and CD, in 10 made solutions, were 0.08 mg mL^{-1}, 0.50 μg mL^{-1} and 0.50 mg mL^{-1}, respectively. The maximum expected concentration of BHA was 0.90 μg mL^{-1}.

2.3.7. Ointment sample preparation

1 gram of ointment was transferred to a 25 mL volumetric flask and dissolved with about 20 mL of mobile phase using an ultraconic bath for 15 min, diluted to volume with mobile phase and filtered. The expected concentrations of active compounds LH, DA and CD were 0.80 mg mL^{-1}, 10.00 μg mL^{-1} and 1.60 mg mL^{-1}, respectively. The maximum expected concentration of BHA was 8.00 μg mL^{-1}.

Two milliliter of this solution was diluted to 10.0 mL with mobile phase. The concentrations were 0.16 mg mL^{-1} for LH, 2.00 μg mL^{-1} for DA and 0.32 mg mL^{-1} for CD. The maximum expected concentration of BHA, was 1.60 μg mL^{-1}. Ten solutions were prepared.

2.3.8. Procedure

Three injections (20 μL) of each solution were made into the chromatographic system.

For calibration curve, the average peak area ratio for each dilution was plotted against the concentration of each of the compounds in the solution. All solutions were freshly prepared daily.

3. Results and discussion

A RP-HPLC method for the determination of LH, DA, CD, BHA and HQ was developed and validated. On the basis of preliminary investigations, optimal chromatographic conditions for parallel separation and determination of all five compounds were established.

The proposed RP-HPLC method showed good selectivity that was validated by the fact that no interference due to excipients was detected in the chromatograms produced (Fig. 1).

The compounds eluted, with the retention factor (k), in the following order: CD t_{R1} = 2.40 min (k = 1.05), hydroquinone t_{R2} = 3.40 min (k = 1.86), LH-hydrochloride t_{R3} = 4.10 min (k = 2.50), dexamethasone acetate t_{R4} = 11.70 min (k = 8.94) and BHA t_{R5} = 17.00 min (k = 13.43). The hold up time (t_0) was determined by injecting the mobile phase into the equilibrated chromatographic system. The system suitability parameters as separation factor (α) and resolution (R) achieved were: $\alpha_{HQ/CD}$ = 1.77 ($R_{HQ/CD}$ = 3.96), $\alpha_{LH/HQ}$ = 1.34 ($R_{LH/HQ}$ = 2.76) $\alpha_{DA/LH}$ = 3.58 ($R_{DA/LH}$ = 18.82) and $\alpha_{BHA/DA}$ = 1.50 ($R_{BHA/DA}$ = 10.53).

The minimum level at which every investigated compound can be reliably detected (limit of detection, LOD) and quantified (limit of quantification, LOQ) were determined experimentally. Limit of detection was measured as the lowest amount of analyse that may be detected to produce a response which is significantly different from that of a blank. Limits of detection for LH, DA, CD, BHA and HQ were 0.05, 0.06, 0.05, 0.20 and 0.015 μg mL^{-1}, respectively. Limit of quantification was measured as the lowest amount of analyte that can be reproducibly quantified above baseline noise, for which duplicate injections resulted in a relative standard deviation (RSD) $\leq$3%.

Limits of quantification for LH, DA, CD, BHA and HQ were 0.50, 0.20, 0.40, 0.40 and 0.04 μg mL^{-1}, respectively.

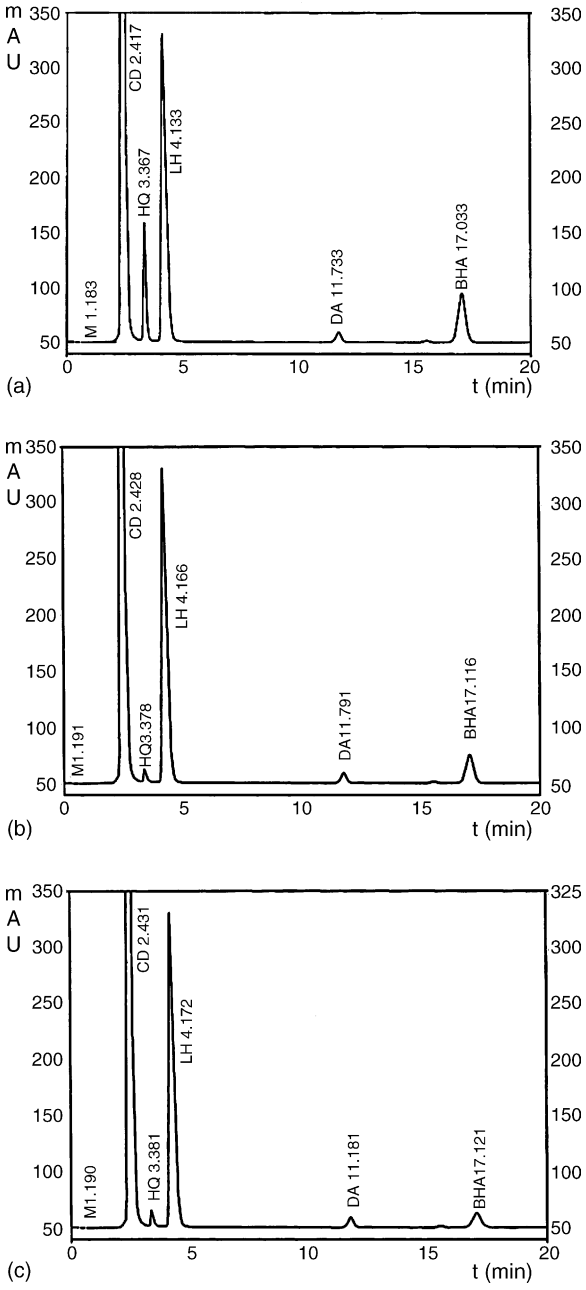

Fig. 1. HPLC chromatogram of system suitability solution of calcium dobesilate (CD), hydroquinone (HQ), lidocaine hydrochloride (LH), dexamethasone acetate (DA) and butylated hydroxyanisole (BHA) bulk drugs (a), in simple of Modolex ointment (b) and in simple of Modolex suppositories (c) mobile phase: acetonitrile:water (50:50, v/v), pH 2.5, flow rate 1.0 mL min^{-1}, column temperature 20 °C and detection at 240 nm (Chanel A for CD, LH and DA) and 290 nm (Chanel B for HQ and BHA) (M—peak corresponding to the mobile phase).

Eight standard solutions were prepared of each compound and series of three injections were made of each concentration to determine the linearity of the response of the system for the five analytes. Regression lines were constructed by the method of least squares with the following regression calibration parameters: LH ($y = 28.15E6x - 0.117E6$, $R = 0.9999$), DA ($y = 0.17E6x - 0.113E6$, $R = 0.9999$), CD ($y = 18.47E6x + 0.297E6$, $R = 0.9994$), BHA ($y = 0.15E6x - 0.112E6$, $R = 0.9998$) and HQ ($y = 0.21E6x - 0.122E6$, $R = 0.9998$) where y is the peak area ratio, x is the concentration of the compound and R is the correlation coefficient.

Table 1
Precision of the assay expressed as the percent RSD of seven samples for two concentrations

Compound	Concentration	Found	SD	RSD (%)	Recovery (%)
CD (mg mL^{-1})	0.32	0.32	0.000*	0.15	100.06
	0.50	0.50	0.001	0.23	100.03
HQ (μg mL^{-1})	1.60	1.58	0.006	0.36	98.99
	5.00	5.01	0.005	0.11	100.12
LH (mg mL^{-1})	0.08	0.08	0.000	0.14	99.99
	0.16	0.16	0.000	0.05	99.98
DA (μg mL^{-1})	0.50	0.50	0.006	1.12	100.98
	2.00	1.99	0.009	0.43	99.96
BHA (μg mL^{-1})	4.00	3.98	0.010	0.25	99.58
	4.50	4.50	0.012	0.26	100.04

* $n = 7$.

The precision of the chromatographic procedure was calculated for two concentrations from the calibration curve of each compound. Seven solutions were made for each known quantity of the analysed compound. The results and important statistical parameters are given in Table 1.

The closeness of the measured value to the true value for the sample (accuracy) was assessed by analyzing a sample of known concentration and comparing the measured value to the true value [17]. Accuracy study were performed in three concentrations of every compound, at 80%, 100% and 120% of label claim for suppositories and ointment, in triplicate [18]. The accuracy results are presented in Tables 2 and 3.

Table 2
Accuracy of the method validation for the determination of the compounds in Modolex Plus ointment

Compound	Concentration	Found	SD*	RSD (%)	Recovery (%)
CD (mg mL^{-1})	0.256	0.2565	0.0003	0.12	100.18
	0.320	0.3196	0.0004	0.13	99.87
	0.384	0.3848	0.0005	0.14	100.21
HQ (μg mL^{-1})	1.28	1.2584	0.0098	0.78	98.31
	1.60	1.5815	0.0205	1.30	98.85
	1.92	1.9112	0.0185	0.97	99.54
LH (mg mL^{-1})	0.064	0.0644	0.0004	0.61	100.69
	0.080	0.0798	0.0003	0.33	99.73
	0.096	0.0960	0.0002	0.17	100.00
DA (μg mL^{-1})	0.40	0.3872	0.0072	1.86	96.79
	0.50	0.4968	0.0109	2.19	99.37
	0.60	0.6075	0.0039	0.65	101.25
BHA (μg mL^{-1})	3.20	3.2217	0.0149	0.46	100.68
	4.00	3.9875	0.0117	0.29	99.69
	4.80	4.7784	0.0181	0.38	99.55

* $n = 3$.

Table 3
Accuracy of the validation method for the determination of the compounds in Modolex Plus suppositories

Compound	Concentration	Found	SD*	RSD (%)	Recovery (%)
CD (mg mL^{-1})	0.400	0.3992	0.0032	0.80	99.79
	0.500	0.5023	0.0017	0.33	100.47
	0.600	0.6010	0.0018	0.30	100.16
HQ (μg mL^{-1})	4.00	4.0304	0.0167	0.41	100.76
	5.00	5.0071	0.0073	0.15	100.14
	6.00	5.9828	0.0189	0.32	99.71
LH (mg mL^{-1})	0.128	0.1277	0.0007	0.58	99.73
	0.160	0.1600	0.0003	0.17	99.97
	0.192	0.1914	0.0004	0.20	99.70
DA (μg mL^{-1})	1.60	1.5740	0.0173	1.10	98.38
	2.00	1.9940	0.0177	0.89	99.70
	2.40	2.4083	0.0174	0.72	100.35
BHA (μg mL^{-1})	3.60	3.6190	0.0123	0.34	100.53
	4.50	4.4955	0.0161	0.36	99.90
	5.40	5.4121	0.0063	0.12	100.22

* $n = 3$.

The RP-HPLC method developed, was used for determination of LH, DA, CD, BHA and HQ in Modolex Plus suppositories and Modolex Plus ointment. Summary of the results of the active compounds content in suppositories and ointment is presented in Table 4.

In suppositories, BHA was found in a concentration of 0.236 mg supp^{-1} with a standard deviation (SD) of 0.003 mg supp^{-1} and a relative standard deviation of 1.28%.

The concentration of HQ, degradation product of CD was 0.131 mg supp^{-1} (SD = 0.004 mg supp^{-1}, RSD = 2.91%) what results in 0.053% of the calculated content of CD.

In ointment BHA was found in a concentration 0.144 mg g^{-1} with standard deviation 0.003 mg supp^{-1} and relative standard deviation 1.97%. The content of hydroquinol was found to be 0.032 mg g^{-1}(SD = 0.001 mg g^{-1}, RSD = 2.39%) what makes 0.081% of the calculated to the content of CD.

Table 4
Parameters of the statistical analysis of results in the determination of content of LH-hydrochloride, dexamethasone acetate and CD in Modolex Plus suppositories and Modolex Plus ointment

Compound	Concentration (mg supp^{-1})	Found	SD	RSD (%)	Recovery (%)
Suppositories					
CD	250.00	253.85	4.249*	1.67	101.54
LH	40.00	39.79	0.277	0.70	99.47
DA	0.250	0.248	0.003	1.20	99.15
Ointment					
CD	40.00	40.58	0.760*	1.88	101.44
LH	20.00	20.10	0.131	0.65	100.49
DA	0.250	0.252	0.002	0.89	100.91

* $n = 7$.

4. Conclusion

The RP-HPLC method proposed allowed separation of five compounds present in suppositories and ointment due to the selectivity of the chromatographic system. The total time of the analysis was not more than 18 min. The good recoveries and acceptable RSD values confirm the proposed RP-HPLC method is applicable and reliable for the determination of LH, DA, CD and BHA in dosage form and its purity. The applied method can be used in quality control as well as purity testing of suppositories and ointments as sensitive, precise and accurate.

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