Scientia Pharmazeutica (Sci. Pharm.) **68**, 189–200 (2000)

Ion-pair Spectrophotometric Determination of Dibucaine

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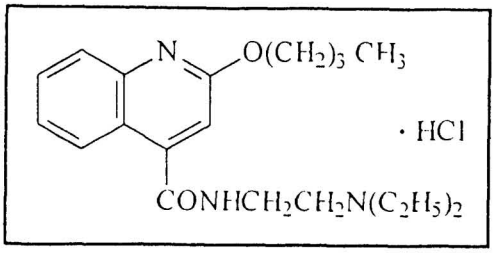
Abstract: A simple and sensitive spectrophotometric determination of dibucain has been established. It is based on the formation of colored ion-pair complexes between Dibucaine and each of Orange II, Orange G, Bromothymol blue and Bromocresol green. The extract with each of the previous dyes in chloroform exhibited a specific wavelength of maximum absorbance, and all these wavelengths lied in the range 416 to 500 nm. The linear range extended from 1.5 to 60 ppm. The optimum conditions were selected after studying many variables such as pH, shaking time, temperature and dye concentrations. The method was also selective for the analyte and the drug excipients did not interfere.

Keywords : Dibucaine, Ion-pair spectrometry

Introduction

Dibucaine [[2-butoxy-N-(2-diethylamino)ethyl]-4-quinolinecarboxamide monohydrochloride] is a local anesthetic drug and was first synthesized by Miescher[1] in 1932. Dibucaine (cinchocaine), $C_{20}H_{29}N_3O_2.HCl$, was one of the famous families, which can be classified as local anesthetic, such as Procaine, Cocaine, Lignocaine and Prilocaine [2,3].

Dibucaine



This drug is about 15 times more potent than procaine and 5 times more potent than cocaine in producing local anesthesia, but it is also considerably more poisonous[4]. It is used for temporary relief of painful sunburn, minor burns, scrapes, scratches, nonpoisonous insect bites, external hemorrhoidal pain, and as an injectable preparation for spinal anesthesia[5,6]. Dibucaine is readily absorbed through the mucous membranes and should not be used around the eyes or mouth. David et al [5] reported a new method for the determination of dibucaine in biological fluids using gas chromatography/mass spectrometer. The detection limit was in the range of 1-80 ng/mL of serum.

Igrashi, *et al* [7] reported a method for determination of Dibucaine and its metabolites in human urine by high performance liquid chromatography with fluorescence detector. Other workers[8]reported an HPLC method also for the drug determination in serum. A single method has been reported in the literature for the electrochemical determination of Dibucaine by its reduction on a mercury electrode(10^{-6} to 10^{-4}M) [9].

Mohamed *et al* [10] reported a spectrophotometric method for the determination of Dibucaine and eight related drugs. Beer's law was obeyed for 5-30 μg/mL Dibucaine and the detection limit was in the range 1-3.7 μg /ml. The aim of our work here is to develop a new spectrophotometric method for the determination of Dibucaine in pharmaceutical formulations. It was based on the formation of ion-pairs

between Dibucaine and the positively charged dyes (e.g. BTB, BCG, OrgG and Org II); the produced colored ion-pair can be extracted to an organic phase and determined spectrophotometrically. The sensitivity and selectivity of the suggested method was compared with other previously reported methods.

Experimental

Absorbance measurements were carried out using a UV-2 UNICAM UV-VIS spectrophotometer with 1-cm quartz cells. The pH measurements were carried out using a (HANNA, model HI 8424) pH- meter. The temperature was controlled using Techne, circulator v-85 A . Dibucaine, Procaine, Prilocaine, Prilocainamide, Orange II, and Orange G were obtained from SIGMA company. Bromothymol blue (BTB) and bromocresol green (BCG) were obtained from BDH. All materials were used without further purification.

Dibucaine stock solution (1.0×10^{-3}M)

A 1.0×10^{-3}M standard aqueous solution of Dibucaine was freshly prepared by dissolving an appropriate amount of pure drug (exactly 0.0379 g Dibucaine) in 100 ml of doubly distilled water in 100-ml volumetric flask. All solutions were prepared weekly and stored in the refrigerator.

Orange II and orange G solutions (1.0×10^{-3}M)

Dye solutions were prepared by dissolving exactly 0.03503g and 0.04524g of

orange II and orange G, respectively in a 100-ml doubly distilled water. The stock solutions were freshly prepared every two weeks.

A 1.0×10^{-3}M of each BTB and BCG solutions were prepared by dissolving exactly 0.0624 g and 0.0698 g of BTB and BCG, respectively in 2 ml of 0.1 M sodium hydroxide, followed by the addition of 20 ml ethanol (96%) then the volume was completed to 100 ml using doubly distilled water.

Recommended procedure

A measured amount of dye (4.0×10^{-5}M in the case of orange II, BTB and BCG, and 8.0×10^{-5}M in the case of orange G) was transferred into a 100-ml separatory funnel, then 5ml of BR-buffer solution of the desired pH were added. An accurate amount of solution containing Dibucaine in the range 1.5-60 μg/ml was added and followed by 20 ml of organic solvent. The mixture was shaken vigorously for 30 - 60 s and allowed separating into two phases. The organic phase was collected in a 25-ml volumetric flask and the volume was completed to the mark with the organic solvent.

Blank solution was prepared at the same time under the same conditions. The absorbance of the formed complex was measured at 486, 500, 418 and 416 nm wavelengths for orange II, orange G, BTB and BCG, respectively. The amount of Dibucaine was determined from the already constructed calibration curves.

Results and discussion

Effect of pH and solvents

The effect of pH of the aqueous phase on the extraction efficiency of Dibucaine ion-pairs were studied in the pH range 1.7-10.0 for solutions prepared as described in the general procedure. The results obtained indicated that the absorbance of the organic phase for all the systems was maximum within the pH range 1.7 – 4.0. A further increase in the pH affected a remarkable decrease in the absorbance. Dibucaine ion-pair complexes with OrgII, OrgG, BTB and BCG showed absorption maxima at 486, 500, 418 and 418 nm, respectively. In the present work, pH 1.75 was selected as optimum for further work when using OrgII and OrgG, and pH 3.00 when using BTB and BCG.

In the proposed methods, chloroform was found to be the most suitable for extraction of Dibucaine-OrgII and Dibucaine-OrgG ion-pairs, while dichloromethane was found to be the most suitable for extraction of Dibucaine-BTB and Dibucaine-BCG ion-pairs. A single extraction for 60 s with 20 ml organic solvent was found to have excellent extraction efficiency. BR buffer gave maximum absorbance and was chosen as optimum. The stability of the ion-pair complexes in the organic layer was determined by measuring the absorbance over a period of time. The colour of the

Dibucaine-dye ion-pair developed instantly and the intensity remained almost constant for at least 120 hours.

Table (1)

Effect of type of organic solvent on the percent of extraction of Dibucaine in presence of OrgII, OrgG, BTB, and BCG dyes at the optimum conditions.

Organic Solvent	Orange II		Orange G		BTB		BCG	
	A	%E	A	%E	A	%E	A	%E
Chloroform	**0.811**	**100**	**0.422**	**100**	0.724	94.4	0.709	91.9
Dichloromethane	0.789	97.3	0.314	74.4	**0.767**	**100**	**0.771**	**100**
Toluene	0.404	49.9	0.052	5.9	0.508	66	0.598	77.6
Diethylether	0.046	7.9	0.008	1.9	0.528	68.8	0.599	77.7
Carbon tetrachloride	0.012	1.5	0.104	24.6	0.743	96.8	0.592	76.7
n-hexane	0.010	1.2	0.009	2.1	0.580	75.6	0.001	0.13

[Dibucaine]=[OrgII]=[BTB]=[BCG]= 4.0×10^{-5}M, [OrgG]= 8.0×10^{-5}M.

Temp. =25 °C, pH = 1.75 for OrgII, OrgG and 3.00 for BTB, BCG.

Interference

The effect of various pharmaceutical excipients and similar compounds from the family of Dibucaine on the determination of 4.0×10^{-5} M of dibucaine was investigated. These included Procaine, Procainamide, Prilocaine, and other excipients

e.g. starch, talc powder, lactose, glucose, and magnesium stearate in the range of 4.0×10^{-7} - 4.0×10^{-5} M.

It was found that the presence of excipients did not exhibit any significant interference in the determination of Dibucaine. On the other hand, in presence of OrgG dye, 4.0×10^{-5}M of each Procainamide and Prilocaine. A significant interference has been observed in the presence of small amounts of Procaine, Procainamide, and Prilocaine (4.0×10^{-5} M) in case of BTB dye.

Beer's law and sensitivity

The calibration graphs were plotted under the optimum conditions recommended in the general procedure. The graphs obey Beer's law (Linear relationship between the absorbance and the Dibucaine concentration) over the concentration range 0.8 – 15 ppm, 3.7-61ppm, 1.5-17ppm and 1.5 –18 ppm for Orange II, Orange G, BTB, and BCG systems, respectively. Fig 1 shows this data for orange II dye complex with Dibucaine. The Molar absorptivities (ε) were calculated from the linear part of the curve and found to be 2.0×10^{4} L mol^{-1} cm^{-1}, 5.7×10^{3} L mol^{-1} cm^{-1}, 1.6×10^{4} L mol^{-1} cm^{-1}, and 2.2×10^{4} L mol^{-1} cm^{-1} for Orange II, Orange G, BTB, and BCG systems, respectively. The relative standard deviations (RSD) were calculated for each of the above ion-pair complexes, and found to be 0.71% and 0.65% for Orange II and Orange G, respectively, and 0.55 % for BTB and BCG. These values indicated the good reproducibility of the proposed methods.

The composition of the ion-pair complexes between Dibucaine and the investigated dyes were determined using the molar ratio method[11]. In this method, a series of solutions containing different concentrations of the dye were prepared. The concentration of Dibucaine and all other conditions were kept constant. The absorbance of the formed complexes were measured at λ_{max} for each complex and plotted versus the mole ratio dye/Dibucaine. Figure 2 indicates that the ion-pair complexes have a 1:1 stoichiometric ratio.

Table (2)

Analytical characteristics for the ion-pair formation methods

Parameter	Org II method	Org G method	BTB method	BCG method
λ_{max} (nm)	486	500	418	416
Amount of buffer (ml)	5	5	5	5
Recommended optimum pH	1.75	1.75	3.00	3.00
Shaking time (s)	60	30	30	60
Concentration of dye (M)	4.0×10^{-5}	8.0×10^{-5}	4.0×10^{-5}	4.0×10^{-5}
Molar absorbtivity(L mol^{-1} cm^{-1})	2.0×10^{4}	5.68×10^{3}	1.6×10^{4}	2.2×10^{4}
Detection limits ($\mu g\ ml^{-1}$)	0.8	3.8	1.5	1.5
Range of linearity ($\mu g\ ml^{-1}$)	0.8-19.0	3.8-60.8	1.5-16.7	1.5-18.2
RSD (n=5)	0.72%	0.66%	0.55%	0.55%

Comparison of the proposed method with other published methods

Very few spectrophotometric and chromatographic methods and only a single electrochemical method have been reported for the determination of Dibucaine in pharmaceutical formulations. It was found that the suggested method is highly sensitive and competes well with other methods. Table 3 shows comparison between the proposed method and other reported methods.

Determination of Dibucaine in Sheriproct Ointment

An ointment called Sheriproct, manufactured in Madrid-Spain, was obtained and analyzed as mentioned in the recommended procedure. OrgII dye was used at pH 1.75. After extraction with 25 ml of chloroform, a solution of this ointment containing 30 μg Dibucaine/ml. The absorbance was then measured at 486 nm versus a similar preparation without ointment (blank). From the calibration graph, it was observed that the assay has a minimum of 93% and a maximum of 96% of the labeled amount. The RSD for six different measurements at the same level was about 1.5%.

Conclusion The use of the proposed method for quantitative determination of Dibucaine allows a selective and quantitative analysis. The results also showed that there is no significant interference from many of the excipients and similar drugs from the same family. The method is applicable to the analysis of trace amounts of Dibucaine in pharmaceutical formulations with a satisfactory reproducibility.

Table (3)

Comparison of the proposed method with existing methods for determination of Dibucaine

Reagent	λ_{max} (nm)	Linear range	Molar absorbtivity L mol^{-1} cm^{-1}	Technique	Reference
TBPE	555	n	2.0×10^{4}	Visible Spect.	12
Serum	_	1-80 ng ml^{-1}	n	GC/MS	5
NaOH, methanol	_	20-2000 ng ml^{-1}	n	GC	13
Methanol, TEA	440,330	50-500 ng ml^{-1}	n	HPLC	7
$CHCl_3$	_	1-100 nM	n	Electrochemical	9
Org II	486	0.75-15 ppm	2.0×10^{4}	Visible Spect.	present work
Org G	500	3.8-60 ppm	5.7×10^{3}	Visible Spect.	present work
BTB	418	1.5-15 ppm	1.6×10^{4}	Visible Spect.	present work
BCG	416	1.5-13.7 ppm	2.2×10^{4}	Visible Spect.	present work

n = not available

TEA= triethylamine

TBPE = tetrabromophenolphthalein ethyl ester

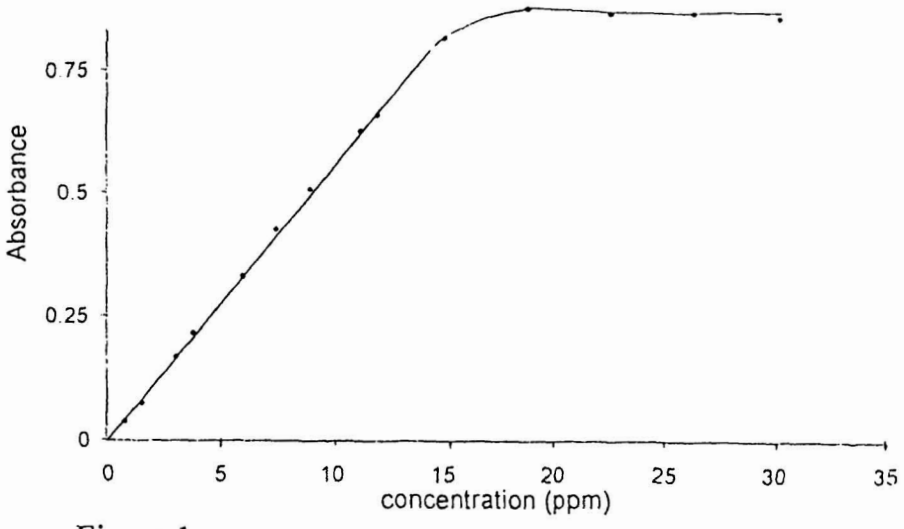


Figure 1

Calibration graph for the determination of Dibucaine using OrgII, with conccentration of OrgII = 4.0×10^{-5} M, pH= 1.75 at 486 nm.

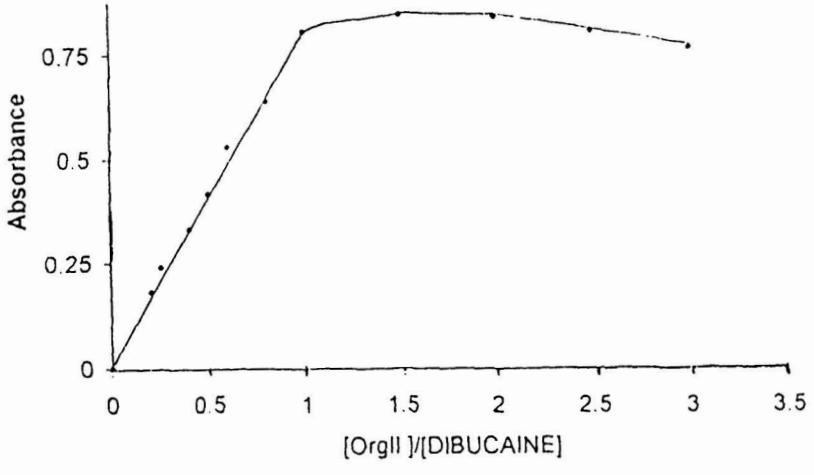


Figure 2

Molar ratio method for the determination of the stoichiometry of the ion-pair formed between Dibucaine and OrgII at 486nm.

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Received December 12th, 1999
Accepted May 4th, 2000