Application Bulletin

Of interest to:	Organic chemistry; Pharmaceutical industry; Biochemistry, biology, medicine	B 3, 4, 8

Polarographic determination of cinchocaine (dibucaine) in pharmaceutical preparations

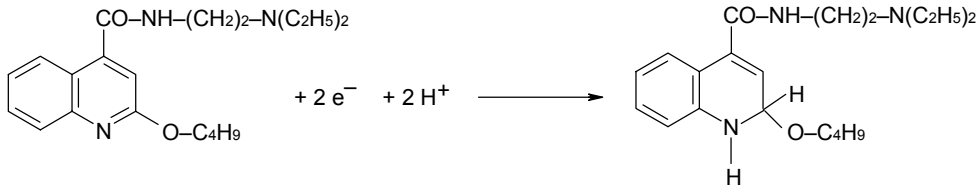
Summary

Cinchocaine (dibucaine) is used in the form of ointments or injection solutions as a local anaesthetic. Its base is soluble in diethyl ether; its hydrochloride, on the other hand, is insoluble in diethyl ether but easily soluble in water.

This bulletin describes the determination of cinchocaine in ointments, creams and injection solutions by means of differential pulse polarography. An acetate buffer pH = 4.8 is used as the supporting electrolyte. The limit of quantitation and the linear working range of the method are given. The necessary sample preparation steps are also dealt with in this bulletin.

Theory

In 0.1 mol/L acetate buffer pH = 4.8 cinchocaine is reduced by accepting two electrons (as well as two H^+ ions), whereby two distinct polarographic reduction peaks are obtained. The first, which appears at ca. –0.93 V, is used to determine the concentration. The second peak at ca. –1.25 V is often interfered with by other substances or hydrogen generation and is therefore not used. The following reaction equation illustrates the electrochemical reduction of cinchocaine occurring on the mercury drops:



Instruments and accessories

- 746 VA Trace Analyzer with 747 VA Stand
  or
  757 VA Computrace
- Magnetic stirrer
- Centrifuge
- Analytical balance (minimum resolution 0.1 mg)
- Drying cabinet
- Desiccator
- Separating funnel, volumetric flasks, measuring cylinders, graduated and bulb pipettes, beakers

Reagents

Only reagents of the highest purity and ultrapure water are used for the preparation of the solutions.

- Diethyl ether, with a degree of purity suitable for HPLC
- Hydrochloric acid c(HCl) = 1 mol/L
- Sodium hydroxide c(NaOH) = 2 mol/L
- Acetic acid c(CH_3COOH) = 2 mol/L
- Supporting electrolyte: acetate buffer pH = 4.7 ... 4.8:

  The acetate buffer contains 0.1 mol/L each of sodium acetate and acetic acid. If necessary, its pH value is adjusted to 4.8 with c(NaOH) = 2 mol/L or c(CH_3COOH) = 2 mol/L.
- Cinchocaine standard solutions:
  - Stock solution with a concentration of 1000 ppm:

    A sufficient amount of cinchocaine hydrochloride is dried for 5 h in a drying cabinet at 80 °C and then allowed to cool down in a desiccator. 500.0 mg of the reference substance prepared in this way are weighed into a 500 mL volumetric flask, dissolved in ultrapure water and made up to the mark. This solution contains 1000 ppm cinchocaine hydrochloride or 904 ppm cinchocaine base. It is stored in a dark bottle in a cool place (cinchocaine is sensitive to light) and is stable for at least one month.
  - Working solution with a concentration of 250 ppm:

    50.0 mL of the 1000 ppm stock solution are transferred to a 200 mL volumetric flask and made up to the mark with ultrapure water. This solution contains 250 ppm cinchocaine hydrochloride or 226 ppm cinchocaine base. It is also stored in a dark bottle in a cool place and is stable for about one month.

Sample preparation

1. Injection solutions

These contain cinchocaine in dissolved form and can thus be used directly for analysis.

2. Ointments/creams containing cinchocaine base

Most pharmacopoeias describe an extraction with diethyl ether (for the subsequent UV determination). With c(HCl) = 1 mol/L the cinchocaine is re-extracted as hydrochloride into the aqueous phase and then determined.

3. Ointments/creams containing cinchocaine hydrochloride

0.9 ... 1.1 g sample are weighed into a beaker and 20 mL c(HCl) = 1 mol/L as well as a magnetic stirring bar are added. The beaker is covered with a watch glass and the sample is then «extracted» with thorough stirring for 15 min at 65 °C on a magnetic stirrer. After cooling down to room temperature 15 mL ultrapure water are added, the mixture is again thoroughly mixed and then transferred to a 50 mL centrifuge tube. The sample is centrifuged for 20 min at a rotating rate of at least 7500 min^{-1} (10 000 min^{-1} is better) and then cooled down to 5 °C (this facilitates the subsequent steps). After cooling down the HCl phase is removed using a pipette with a finely drawn-out tip (make sure not to pick up any floating or precipitated particles) and transferred to a 100 mL volumetric flask. The pipette is rinsed out into the beaker already used with a little ultrapure water. The contents of the centrifuge tube are also rinsed into this beaker with 10 mL c(HCl) = 1 mol/L and then «extracted» for a second time for 10 min at 65 °C. Afterwards the beaker contents are transferred to the centrifuge tube already used with a little ultrapure water, centrifuged, then the HCl phase is removed and combined with the first extract in the 100 mL volumetric flask. The contents of the flask are made up to the mark with ultrapure water and mixed. The solution is usually still slightly turbid, which, however, does not interfere with the polarographic determination. Strong turbidities can be removed by filtering a portion of the sample solution through a paper filter (do not rinse the filter).

Analysis

1.00 mL ointment extract or a corresponding amount of injection solution is placed in the polarographic vessel, 19.0 mL supporting electrolyte are added and the mixture is purged with nitrogen for 5 min. The polarograms are then recorded under the following conditions:

Method / Amplitude	DP / –25 mV
Electrode	DME or SMDE
U.start	–0.70 V
U.end	–1.20 V
Sweep rate	7.5 mV/s

The peak potential of cinchocaine lies at ca. –0.93 V.

The concentration is determined by two-fold standard addition.

Remarks

- The absolute mass of cinchocaine in the polarographic vessel including the standard additions should not exceed 340 µg (cinchocaine base) or 375 µg (cinchocaine hydrochloride), as these represent the upper limit of the linear working range.
- The limit of quantitation is 2.8 µg cinchocaine base or 3.1 µg cinchocaine hydrochloride per 20 mL. However, this is not of great importance in this case as the pharmaceutical preparations analysed have relatively high active substance concentrations.
- If high-purity reagents are used it is normally not necessary to carry out a blank determination on the chemicals as in comparison with the high cinchocaine content of the samples the blank can be ignored.
- The polarographic determinations using the DME and the SMDE yield comparable results. Due to the smaller mercury drops, considerably lower peak heights are obtained with the SMDE, which, however, consumes much less mercury.

Literature

- US Pharmacopoeia XXI (1984) 310-312.
- J. Volke
  Polarographic and voltammetric methods in pharmaceutical chemistry and pharmacology
  J. Electroanal. Chem. 155 (1983) 7–23.

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==================== METROHM 693 VA PROCESSOR (5.693.0020) ===================
 Method  Dibcream.mth          OPERATION SEQUENCE
 Title   Detn. of Dibucaine in Antiseptic Cream
```

	Instructions	t/s	Main parameters		Auxiliary parameters	
1	SMPL/M		V.fraction	1.000 mL	V.total	0.1L
2	DOS/M		V.added	19.000 mL		
3	PURGE					
4	STIR	300.0	Rot.speed	3000 /min		
5	(ADD					
6	NOP	15.0				
7	SEGMENT		Segm.name	pol		
8	ADD>M		Soln.name	dibstd	V.add	0.100 mL
9	ADD)2					
10	END					

Method: Dibcream SEGMENT
pol

	Instructions	t/s	Main parameters		Auxiliary parameters	
1	(REP					
2	0PURGE					
3	0STIR					
4	DME					
5	OPMODE		U.ampl	-25 mV	t.meas	20.0 ms
			t.step	0.80 s	t.pulse	40.0 ms
6	SWEEP	69.6	U.start	-700 mV	U.step	6 mV
			U.end	-1200 mV	Sweep rate	7.5 mV/s
7	0MEAS					
8	REP)1					
9	PURGE					
10	STIR		Rot.speed	3000 /min		
11	END					

Method: Dibcream DOCUMENTATION

Auto form feed no

COPY Reports, Curves	TO Destination
Curve Smth Sbst:dibuc VR:**	Printer
Report Full	Printer
Report MethSpc	Printer

Fig. 1: *Method for the polarographic determination of cinchocaine in cream (performed on the 693 VA Trace Analyzer): Operation Sequence, Segment and Documentation.*

Method: Dibcream SUBSTANCES
dibuc - pol

Recognition

U.verify	-935 mV
U.tol (+/-)	30 mV
U.width min	10 mV
U.width max	200 mV
I.threshold	250 pA

Display / Plot

I.scale	auto
U.div	50 mV/cm
U.begin	-700 mV
U.end	-1200 mV

Baseline

Type	linear
Scope	whole
dU front	auto
S.front	auto
dU.rear	auto
S.rear	auto

Evaluation (for peaks only)

Quantity	I.peak

Calibration 94-01-14 14:33

Technique	std.add.
Curve type	linear

Coefficients

Y.reg	-5.276e-08
Slope	-2.111e-05
Nonlin.	
Mean dev.	9.756e-10

Additions

Soln.name	dibstd			
Mass conc.	226 mg/L	g/L	g/L	g/L
Range min	g/L	g/L	g/L	g/L
Range max	g/L	g/L	g/L	g/L
M.conc./cm	g/L	g/L	g/L	g/L

Method: Dibcream CALCULATION
max. 15 lines

Quantity	Formula (R##, C##, A##)	Res.unit	Sig.dig.
dibuc	R1000=MC:dibuc * Vtot * 100/S0	%	5

Fig. 2: *Method (continued): Substances and Calculation.*

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====================== METROHM 693 VA PROCESSOR (5.693.0020) ====================
Determ.      :  01141915            User:                        Date:  94-01-14
Modified     :  no                  Run  :  1                    Time:  14:23:43
Sample table:  -
--------------------------------------------------------------------------------
 Pos.  Ident.1/S1     Ident.2/S2     Ident.3/S3     Method.call   Sample size/S0
       nupercainal          1.000            1.0                        1.0438 g
--------------------------------------------------------------------------------
Method   :  Dibcream
Title    :  Detn. of Dibucaine in Antiseptic Cream
Remark1  :  Pharm. Prod. - Nupercainal Cream - 0.5% w/w Dibucaine
Remark2  :  ca.1.0g-USP prepn.-aliquot 1.0 mL/100mL. 2adds/1rep.
--------------------------------------------------------------------------------

 Substance :  dibuc                                              Comments
 Mass conc.:     49.99 mg/L              Mass      :  49.99 ug   ----------
 MC.dev.   :     0.803 mg/L (1.61%)      Add.mass  :  22.6 ug
 Cal.dev.  :     -                       V0.sample:     1 mL

              VR   U/mV     I/nA    I.mean  Std.dev.  I.delta   Comments
              ---  ------   ------  ------  --------  --------  ----------
              00   -926     -53.16  -52.80   0.5007
              01   -926     -52.45
              10   -927     -75.64  -76.15   0.7213   -23.35
              11   -927     -76.66
              20   -927     -99.47  -99.52   0.0710   -23.37
              21   -927     -99.57

Substance   Techn.     Y.reg/offset    Slope        Nonlin.     Std.add.mass
---------   --------   ------------    ---------    --------    ------------
dibuc       std.add.     -5.276e-08    -2.111e-05                   22.6 ug

                              SOLUTIONS
                               max. 40
================================================================================

 Soln.name    Pos.    Std.subst.    Mass conc.        Remark
 ----------   -----   ----------    ----------        ------
 dibstd         -     dibuc            226 mg/L


C#  Workg.com.var  Remark
--  -------------  ------

Final results                        +/- Res.dev.    %      Comments
-------------                        ------------    ---    --------
     dibuc =  0.47892 %                  0.008      1.61
```

Fig. 3: *Full report for the determination of cinchocaine in cream.*

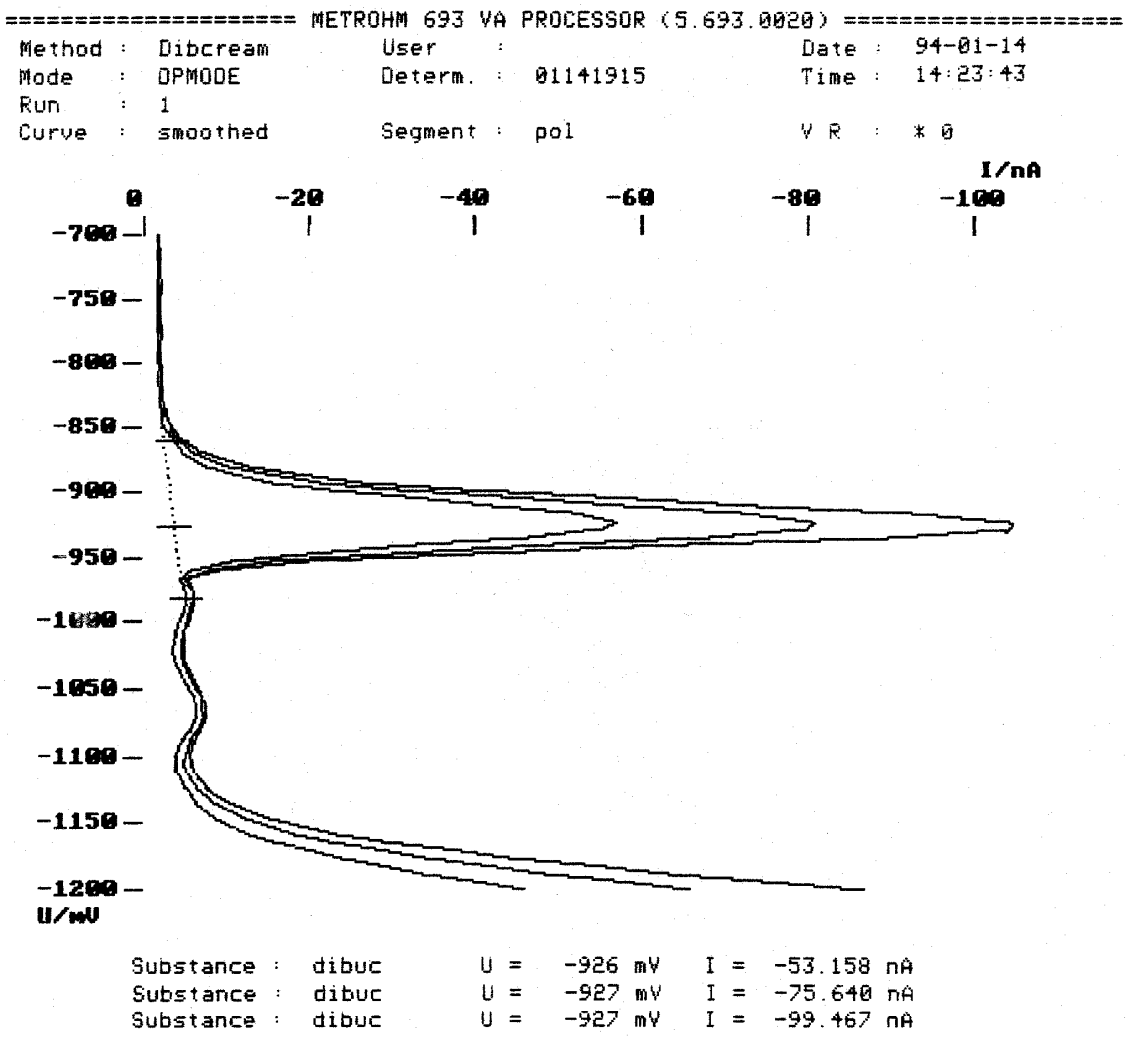

Fig. 4: *Polarograms for the determination of cinchocaine in cream.*