Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Germany

A convenient TLC method for the identification of local anesthetics

M. SCHMIDT, F. BRACHER

Dedicated to Prof. Dr. K. Görlitzer, Braunschweig, on the occasion of his 65th birthday

Received April 25, 2005, accepted May 3, 2005

Prof. Dr. Franz Bracher, Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstr. 5–13, 81377 München, Deutschland
Franz.Bracher@cup.uni-muenchen.de

Pharmazie 61: 15–17 (2006)

A TLC method for the separation of seven local anesthetics and the related antiarrhythmic drug procainamide was developed. Spraying the plates with cobalt(II) thiocyanate solution, followed by spraying with Ehrlich's reagent allowed a clear distinction between the drugs, except for the couple articaine/prilocaine. Articaine could be distinguished from prilocaine and other local anesthetics by a colour reaction with copper(II) sulphate solution.

1. Introduction

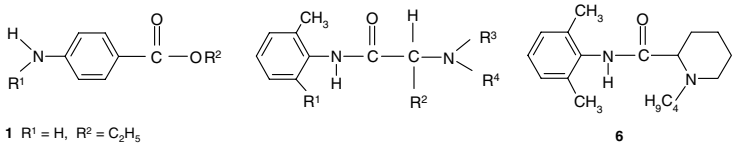
Local anesthetics are a group of drugs mainly from two structural classes, the 4-aminobenzoic esters, and the acylanilides. Benzocaine (**1**), procaine (**2**), and tetracaine (**3**) belong to the first group, lidocaine (**4**), prilocaine (**5**), and bupivacaine (**6**), as well as the thiophene analogue articaine (**7**) to the second group. We also included the structurally related antiarrhythmic drug procainamide (**8**), a 4-aminobenzoic amide, into our investigations.

This project was aimed at the clear distinction between the drugs mentioned above by methods that can also be applied in pharmacies. So we selected TLC techniques combined with detection with appropriate reagents. Several reports dealing with this problem have been published before (Baker and Gough 1979; O'Neal et al. 2000; Deakin 2003). Most of these methods were initiated by the current problem of fast and unambiguous identification of cocaine in drugs of addiction using simple field tests. Frequently, illegal cocaine preparations are adultered with uncontrolled local anesthetics (Baker and Gough 1979). In the field tests it is desirable to have colour reagents for the detection of cocaine without interference with the local anesthetics. The most common reagent for identification of cocaine is cobalt(II) thiocyanate solution (Scott's reagent), but it had been shown earlier, that some of the local anesthetics and numerous other amines also give positive reactions. The coloured products are estimated to have the formula (amine-H)$_2$[Co(SCN)$_4$] in acidic, and [Co(amine)$_2$](SCN)$_2$ in slightly acid to neutral solutions (Stainier 1974). The Ph. Eur. uses a cobalt(II) thiocyanate reagent for the identification of macrogols and polysorbates. This encouraged us to investigate the application of Scott's reagent for the identification of a broad series of local anesthetics.

2. Investigations and results

2.1. TLC separation

In order to avoid time consuming sample preparations we intended to find a TLC eluent that allows direct application of either free amines or hydrochlorides. For this purpose the eluent had to be either acidic (in order to obtain *in situ* protonation of the amines) or alkaline (to liberate the free amines from the salts). First experiments with acidic eluents, e.g. the acetic acid/hexane/dibutyl ether



1 R^1 = H, R^2 = C_2H_5

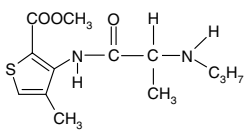
2 R^1 = H, R^2 = $CH_2CH_2N(C_2H_5)_2$

3 R^1 = C_4H_9, R^2 = $CH_2CH_2N(CH_3)_2$

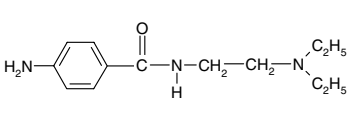
4 R^1 = CH_3, R^2 = H, R^3, R^4 = C_2H_5

5 R^1 = H, R^2 = CH_3, R^3 = H, R^4 = C_3H_7

6



7



8

Table: R_f values of the drugs, detection on TLC, and colour reaction with copper(II) sulphate

Drug	R_f value	relative R_f value*	TLC; detection with TLC; subsequent		alkaline Cu^{2+} in a test tube
			$Co(SCN)_2$	detection with Ehrlich's reagent	
1	0.92	2.42	–	orange	–
2	0.57	1.50	pale blue	orange	–
3	0.38	1.00	deep blue	yellow**	–
4	0.84	2.21	blue	–	blue
5	0.78	2.05	–	–	reddish-brown
6	0.88	2.32	deep blue	–	–
7	0.78	2.05	–	–	brownish-green
8	0.08	0.21	–	orange	–

* relative R_f values calculated on tetracaine (**3**) = 1.00
** no colour without prior spraying with the $Co(SCN)_2$ reagent

mixture used by Ph. Eur. for the impurity test of procaine hydrochloride gave disappointing results. Next we investigated eluents containing various amounts of diethylamine. These eluents gave moderate to good separation of the drugs **1** to **8**. However, some of the peaks appeared in a distorted shape due to the formation of a β-front (Rücker et al. 1992) on TLC.

This problem could be avoided by substitution of ammonia solution for the amine (compare the diethyl ether/methanol/ammonia mixture used in Ph. Eur. for the analysis of prilocaine). A mixture of ethyl acetate/methanol/ammonia solution (32%) was suitable for the separation of the drugs. Even the hydrochlorides gave sharp spots provided that freshly prepared eluent was used. Developing a second TLC plate with the same eluent system resulted in tailing of the drugs that had been administered as hydrochlorides. The Table shows the R_f values of the drugs, and the realative R_f values calculated on the standard tetracaine. Articaine (**7**) and prilocaine (**5**) were not separated by this eluent system.

This initiated us to investigate various detection reagents for the identification of drugs having similar R_f values on TLC.

2.2. Colour reagents for detection on TLC

First experiments with the cobalt(II) thiocyanate reagent, prepared from cobalt(II) nitrate and ammonium thiocyanate, on a spotting tile showed that all local anesthetics give deep blue precipitates. To our surprise, things were different when the reagent was used for detection on TLC plates. Tetracaine (**3**) and bupivacaine (**6**) reacted spontaneously giving deep blue spots, lidocaine (**4**) gave a blue, and procaine (**2**) a pale blue colour, whereas the other drugs did not give coloured spots. The spots should be identified immediately, since the TLC plates become all blue within a few hours. After prolonged storing of the plates, the spots can be visualized again by spraying the plates with water (Lane 1965). Both cobalt(II) nitrate solution (compare identification E of lidocaine (**4**) in Ph. Eur.) and ammonium thiocyanate solution failed to give coloured spots.

Since the drugs **1** and **2** and **8** represent primary aromatic amines, we also investigated Ehrlich's reagent (4-dimethylaminobenzaldehyde in dilute HCl) for detection. As expected, we obtained orange spots for **1**, **2**, and **8** with this reagent. Surprisingly, somewhat different results were obtained in the case we first sprayed with cobalt(II) thiocyanate reagent and then with Ehrlich's reagent. Once again the three primary amines gave orange spots, but in addition, tetracaine (**3**) gave a bright yellow spot.

Taking the different R_f values and the two detection methods into account, the drugs could unambiguously be identified, with exception of the couple prilocaine (**5**)/articaine (**7**).

2.3. Distinction between prilocaine and articaine

Since the TLC system did not enable us to distinguish between **5** and **7**, we looked for further colour reactions that should solve this problem. Articaine (**7**) contains an ester group, and so we first examined the hydroxamic acid reaction (N. N. 1984), but **7** failed to give the expected coloured iron(III) chelate both on TLC and in a test tube. Next, we used isatine/sulphuric acid, a reagent that had been described to give coloured condensation products ("indophenines") with thiophenes (Curtis and Philipps 1962). However, on treating with the isatine reagent on a spotting tile, not only articaine (**7**), but also the other drugs gave untypical yellow to orange colours. Finally, we found that the desired distinction can be performed by a colour reaction in a test tube. Dissolving the drugs in dilute hydrochloric acid, followed by addition of copper(II) sulphate solution and a slight excess of sodium hydroxide solution (Kovar and Ruf 1998) gave a clear reddish-brown solution with prilocaine (**5**) and its hydrochloride, whereas articaine (**7**) gave a brownish-green solution. Lidocaine hydrochloride (**4**) gave a deep blue solution (Vinkler et al. 1978; also compare the identification of lidocaine hydrochloride in the Austrian Pharm. 1960), and the other drugs only gave light blue clear or opalescent solutions. Unfortunately, this colour reaction could not be applied for detection of the spots on TLC.

3. Discussion

In conclusion, we have worked out a convenient method for the identification and distinction of seven local anesthetics and the related antiarrhythmic drug procainamide using TLC with a combination of two detection reagents (cobalt(II) thiocyanate solution; Ehrlich's reagent). Articaine (**7**) and prilocaine (**5**), the only two drugs that were not separated on TLC, could be distinguished by a colour reaction with copper(II) sulphate solution in a test tube.

4. Experimental

4.1. Drugs

Articaine (3M ESPE, Seefeld), prilocaine and prilocaine hydrochloride (AstraZeneca, Wedel), bupivacaine (Synopharm, Barsbüttel), procaine hydrochloride, lidocaine hydrochloride, and tetracaine hydrochloride (SIGMA), procainamide hydrochloride and benzocaine (Aldrich).

4.2. Detection reagents

4.2.1. Cobalt(II) thiocyanate solution

2.5 g Cobalt(II) nitrate and 7.5 g ammonium thiocyanate were dissolved in 50 ml water.

4.2.2. Ehrlich's reagent

2.0 g 4-Dimethylaminobenzaldehyde were dissolved in a mixture of 55 ml hydrochloric acid (36%) and 45 ml water.

4.2.3. Isatine reagent for thiophenes

0.4 g Isatine were dissolved in 100 ml conc. sulphuric acid.

4.3. TLC separation

TLC plates: Merck Kieselgel 60 F_{254} plates (0.25 mm; 10 × 10 cm).
Eluent: ethyl acetate/methanol/ammonia solution (32%), 48 : 1:1.5, mixed by vigorous shaking. The eluent was placed in a TLC chamber, the cham-

ber was closed and kept for 15 min at ambient temperature for equilibration. Fresh eluent has to be prepared for any single separation.
About 2 µl of 25 mg/5 ml solutions of the drugs in ethanol were spotted on the TLC plate. The plates were developed at ambient temperature, and then dried in a current of warm air.
Detection: a) UV 254 nm; b) spraying with cobalt(II) thiocyanate solution; c) subsequent spraying with Ehrlich's reagent.

4.4. Colour reaction with copper(II) sulphate

The drugs (2–3 mg) were suspended in 1 ml water in a test tube and dissolved by addition of 5 drops of 2 N hydrochloric acid. If necessary, the test tube was shaken until the drug was completely dissolved. Then 3 drops of a 2% copper(II) sulphate solution were added, followed by 7 drops of a 2 N sodium hydroxide solution.

This work was performed as part of the practical course "Wahlpflichtpraktikum" for pharmacy students.

References

Baker PB, Gough TA (1979) The rapid determination of cocaine and other local anesthetics using field tests and chromatography. J Forensic Sci 24: 847–855.
Curtis RF, Phillips GT (1962) Thin-layer chromatography of thiophene derivatives. J Chromatogr 9: 366–368.
Deakin AL (2003) A study of acids used for the acidified cobalt thiocyanate test for cocaine base. Microgram J 1: 40–43.
Kovar K-A, Ruf COL (1998) Auterhoff/Kovar: Identifizierung von Arzneistoffen, 6. ed.. Wissenschaftliche Verlagsgesellschaft, Stuttgart.
Lane ES (1965) Thin-layer chromatography of long-chain tertiary amines and related compounds. J Chromatogr 18: 426–430.
N. N. (1984) Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie, E. Merck, Darmstadt.
O'Neal CL, Crouch DJ, Fatah AA (2000) Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Sci Intern 109: 189–201.
Rücker G, Neugebauer M, Willems GG (1992) Instrumentelle pharmazeutische Analytik. Wissenschaftliche Verlagsgesellschaft, Stuttgart.
Stainier C (1974) L'utilisation du thiocyanate de cobalt dans l'analyse des bases organiques. Analyst 97: 263–265.
Vinkler E, Klivényi F, Csŭkonyi VK (1978) Zur Identitätsreaktion des Lidocains und einer neuen spektrofotometrischen Bestimmung. Pharmazie 33: 379.