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New method for the quantification of dequalinium cations in pharmaceutical samples by absorption and fluorescence diode array thin-layer chromatography

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ABSTRACT

A diode array HPTLC method for dequalinium chloride in pharmaceutical preparations is presented. For separation a Nano TLC silica gel plate (Merck) is used with the mobile phase methanol—7.8% aqueous $NH_4^+CH_3COO^-$ (17:3, v/v) over a distance of 6 cm. Dequalinium chloride shows an R_F value of 0.58. Pure dequalinium chloride is measured in the wavelength range from 200 to 500 nm and shows several by-products, contour plot visualized in absorption, fluorescence and using the Kubelka–Munk transformation. Scanning of a single track in absorption and fluorescence measuring 600 spectra in the range from 200 to 1100 nm takes 30 s. As a sample pre-treatment of an ointment it is simply dissolved in methanol and can be quantified in absorption from 315 to 340 nm. The same separation can also be quantified using fluorescence spectrometry in the range from 355 to 370 nm. A new staining method for dequalinium chloride, using sodium tetraphenyl borate/HCl in water allows a fluorescence quantification in the range from 445 to 485 nm. The linearity range of absorption and fluorescence measurements is from 10 to 2000 ng. Sugar-containing preparations like liquids or lozenges with a reduced sample pre-treatment can be reliably quantified only in fluorescence. The total for the quantification of an ointment sample (measuring four standards and five samples), including all sample pre-treatment steps takes less than 45 min!

1. Introduction

Dequalinium chloride [decamethylenebis(4-aminoquinaldinium) chloride] is an anti-bacterial and anti-fungal agent, active against many Gram-positive and Gram-negative bacteria and against *Borrelia vincenti*, *Candida albicans* and some species of *Trichophyton*. It is used pharmaceutically in the form of ointments, as liquids or as lozenges in the treatment of infections of the mouth and the throat [1].

In the official Ph. Eur. dequalinium chloride is quantified using a titration method [2]. An HPLC/UV method is recommended to test the purity, detecting the compound at 240 nm. Three different impurities are described [(e.g. 4-amino-1-(10-4′-quinaldinylaminodecyl)quinaldinium chloride, 4-aminoquinaldine)] [2–6]. If TLC is used for purity checking with the mobile phase butanol, formic acid water (45:10:30, v/v) on silica gel G, dequalinium chloride (R_F = 0.23) and several side spots are visible [7].

Commonly, dequalinium chloride is quantified using HPLC [3,4,6,8–11] or capillary electrophoresis [12,13]. Only in very few publications thin-layer chromatography is used to separate dequalinium chloride [4,5,7,14]. Only one TLC publication describe the attempt of a dequalinium chloride quantification in pharmaceutical products [14]. In this article quartenary ammonium compounds like cetylpyridinium chloride, benzalconium chloride e.g. are quantified in pharmaceutical preparations. In particular dequalinium chloride is separated on silica gel 60 F254, with methanol–8% aq. ammonium acetate (19:1) or ethyl acetate–methanol–anhydrous acetic acid (5:13:5, v/v/v) as mobile phase. After separation the plate is densitometricly evaluated at 240 nm and after additional spraying with Dragendorff reagent the plate is evaluated at 360 nm. Surprisingly, TLC is not used for the quantification of dequalinium chloride in pharmaceutical preparations, although our experience with HPLC and CE has convinced us to see HPTLC as the method of choice for dequalinium chloride quantifications.

The purposes of this article are to publish an HPTLC method, suitable to quantify dequalinium chloride in pharmaceutical preparations. Secondly it should be shown that HPTLC with diode array detection (DAD) in conjunction with a modern theory of evaluations in scattering media [15] is a versatile and reliable working

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method which shows some advantages in comparison to HPLC or CE.

2. Theory

In planar chromatography light is used for detecting separated sample spots by illuminating the TLC or HPTLC plate from the top with light of known intensity (I_0). If the illuminating light shows higher intensity than the reflected light (J), a fraction of light must be absorbed by the sample (the analyte) and/or the TLC layer. The definition of the total absorption coefficient a, which describes plate and sample light absorptions, is:

$$I_{abs} = I_0 - J = aI_0 \quad (1)$$

Theoretical considerations lead to the following equation for transformation purposes, showing linearity between the transformed measurement data (TMD) and the absorption coefficient [15].

$$\text{TMD}\ (\lambda) = k\left(\frac{I_0}{J} - \frac{J}{I_0}\right) + \left(\frac{J}{I_0} - 1\right) = \frac{a}{1-a} \quad (2)$$

where k is the backscattering factor ($k \geq 0$ and $k \leq 1$), I_0 the illuminating light intensity at different wavelengths, J the intensity of reflected light at different wavelengths, and a the total absorption coefficient.

For $k = 1$, Eq. (2) describes a situation where all the light is reflected from the plate surface. (In comparison with the incident light all the light is reflected "backward"). No inner parts of the TLC layer are illuminated and light absorption occurs only at the layer surface. With $k = 1$ (which describes the "backward-scattering"), Eq. (3) can be derived from Eq. (2).

$$\text{TMD}\ (\lambda, k = 1) = \left(\frac{1}{R} - 1\right) = \frac{a}{s} \quad (3)$$

where R is the reflectance ($R = J/I_0$), and s the scattering coefficient ($s = 1 - a$).

Eq. (3) transforms light-losses caused by absorptions into positive values [15]. In Fig. 1, the amount of 2 μg of pure dequalinium chloride (>90% purity) is separated on a HPTLC plate, scanned with a DAD scanner and evaluated using Eq. (3).

The contour plot comprises the measurement data of a single track at different wavelengths. To measure a contour plot, a track of a TLC plate is scanned by use of a diode array detector. Usually the plate is moved below an interface which illuminates the plate at different wavelengths and detects the reflected light. For each wavelength the reflected light intensity (J) and the light intensity (I_0) of the illuminating lamp are measured. A contour-plot comprises the TMD data at different wavelengths and different track locations. These spectral data can be used to extract the UV–vis spectra of different zones as well as the fluorescence spectra. The wavelength range for quantification evaluations can be extracted from the absorption or fluorescence spectra.

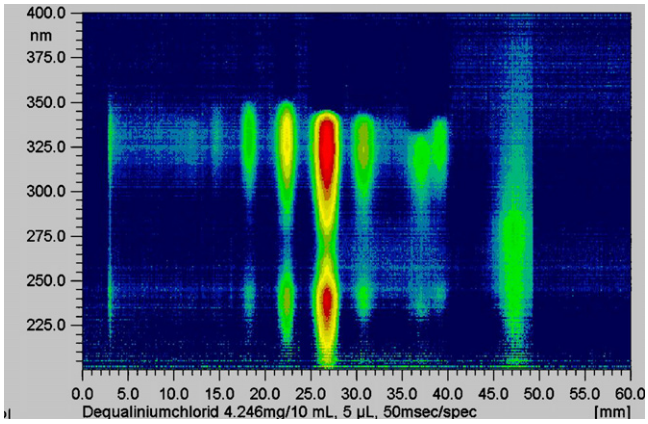

Fig. 1. Contour plot in absorption of a dequalinium chloride separation in the wavelength range from 200 to 400 nm over a separation distance of 50 mm. The measurement time for the whole track is 30 s.

In Fig. 1, the signal of dequalinium chloride can be seen at 27 mm separation distance. Sample application was done at 3 mm and the mobile phase moved to 50 mm. At 16, 18, 23, 31, 37 and 39 mm distances side peaks are detectable showing similar spectral distributions of dequalinium chloride. In summary, more than six signals of side compounds are detectable.

For $k = 0$ no incident light is reflected on the plate top [15]. All light is scattered "forward", in the direction of the incident light. Light leaving the TLC plate at the top must therefore be fluorescent light.

$$\text{TMD}\ (\lambda, k = 0) = (R - 1) \quad (4)$$

A contour-plot evaluated using the fluorescence formula instantly reveals fluorescing compounds at the track.

In general, fluorescence measurements are more specific than absorption measurements, due to the restricted number of compounds showing fluorescence. Dequalinium chloride shows a very bright fluorescence which is induced even by the light of a simple deuterium lamp. Therefore light absorptions and fluorescence can be registered simultaneously. Evaluations in both measurement modes will make a quantification result more reliable.

The Kubelka–Munk theory was first published in the year 1931 and is based on the assumption that half of the scattered flux is directed forwards and half backwards [16–18]. With $k = 1/2$ the Kubelka–Munk expression results from Eq. (2):

$$\text{TMD}\ (\lambda, k = 1/2) = \frac{(1-R)^2}{2R} = \frac{a}{s} \quad (5)$$

Kubelka–Munk transformed date show a linear relationship between measurement data and high sample amounts (>2 μg per zone).

3. Experimental

All the chemicals used were of analytical reagent grade. Dequalinium chloride, benzalkonium chloride as well as sodium tetraphenyl borate were purchased from Fluka (Buchs, Schwitzerland). The purity of dequalinium chloride were 92%. HPTLC plates (10 cm × 10 cm) with the stationary phase silica gel K60 nano HPTLC plates (Merck 5629 with a fluorescent dye) were used. The plates were obtained from Merck (Darmstadt, Germany) as well as ammonium chloride, methanol, ethylene glycol and hydrochloric acid.

Dequonal (liquid used in the treatment of infections of the mouth and the throat) is from Kreussler Pharma (Wiesbaden, Germany) and contains 1.5 mg dequalinium chloride and 3.5 mg benzalkonium chloride in 10 g solution.

Jasimenth lozenges (Lutschpastillen in German) are from Bolder Arzneimittel (Cologne, Germany). A single lozenge contains 0.45 mg dequalinium chloride (and 20 mg ascorbic acid, 70 mg sorbitol, 413 mg sucrose and saccharin sodium).

Evazol ointment is from Ravensberg (Konstanz, Germany) and contains 4 mg dequalinium chloride (and cetylstearyl alcohol and polyethylene glycol) in 1 g ointment.

3.1. Sample preparation

Aqueous Dequonal liquids of dequalinium chloride (d_{20} = 1.0262 g/mL) were applied in an amount of 3.5 μL directly on plate using a very low application velocity (e.g. 1 μL/100 s). The amount of 1.25 g ointment is dissolved in methanol to a