行政院國家科學委員會專題研究計畫　成果報告

化妝品與養髮製品中毛果芸香鹼測定方法之研究

計畫類別：個別型計畫
計畫編號：NSC94-2113-M-041-001-
執行期間：94 年 08 月 01 日至 95 年 07 月 31 日
執行單位：嘉南藥理科技大學醫藥化學系

計畫主持人：王來好

報告類型：精簡報告

處理方式：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中　華　民　國 95 年 10 月 12 日

政院國家科學委員會專題研究計畫成果報告

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中文摘要

利用汞膜修飾金與碳纖維電極，研究金屬(銅、鈷、鎳與鋅)錯合物之伏安行爲。將汞膜修飾金絲電極當做電化學偵測器(electrochemical detector, ECD)之電池的工作電極連接高效能液相層析儀，測定藥物毛果芸香鹼含量。

Abstract

The voltammetric behaviors of metal chelate namely copper, cobalt, nickel and zinc in the presence of pilocarpine on thin-film mercury modified gold and carbon fiber electrodes have been studied. Cobalt (II) forms a labile chelate with pilocarpine in0.1 M lithium perchlorate, which subjected to differential pulse voltammetry at mercury coat–ed carbon fibre and gold wire microelectrodes, respectively, give rise to a catalytic phenomenon which can be utilized for the determination of pilocarpine in the pharmaceuticals. The gold with surface deposit of mercury was used as sensor in liquid chromatography-electrochemical detection (LCEC) to enhance the sensitivity and selectivity of analytical methods for the determination of trace concentration of pilocarpine.

報告內容

前言

Introduction

Imidazole compounds are of considerable biomedical important [1], for example, pilocarpine (2(3H)-Furanone, -ethyldihydro-4-[(1-methyl-1H-imidazol-5-yl) methyl]-) is a cholinergic parasympathomimetic agent belonging to the group of imidazole alkaloids [2]. Recently it has been widely used as a topical agent for the treatment of glaucoma [3] and for the management of patients with xerostomia [4]. The transition metal complexes aromatic N-containing ligands such as imidazole and their derivatives to antitumor agents, is drawing attention [5]. The imidazole nucleus is polarographically inert and the lactone ring of pilocarpine is not reducible at a mercury electrode down to –2.5 V [6]. Although Kolthoff [7] reported a catalytic wave for pilocarpine at –1.8 V (pH 8), it was found to be of little value. The polarographic (voltammetric) effects of the evolution of hydrogen catalysed by ligands in solutions containing cobalt and nickel salts are very important for a sensitive analytical determination [8-10]. Review the literature concerns to the authors' recent research on the reduction of Ni (II) and Co (II) at mercury electrodes

catalysed by imidazole compounds [11-14]. Complexation methods employing copper, iron, nickel and cobalt for electroactive pharmaceuticals have been utilized [15-17]. However, up to the present, there have been relatively few reports of analytical methods for determining polarographically inert substances such as pilocarpine, which complex with metal ions [18-19]. In addition to polarography, the survey of the literature for pilocarpine determination include ion-selective electrode [20], spectrophotometry [21-23], gas-chromatography with mass spectrometric detection [24], liquid chromatography using fluorescence derivation [25], with UV 214 nm [26-27], with tandem with mass spectrometric detection [27], and micellar electrokinetic capillary chromatrography [29-30].

研究目的

The poly (vinyl chloride) pilocarpine membrance electrodes only suited in the analysis of ophthalmic preparations by direct potentiometry. The determination of pilocarpine by conventional spectrophotometry without prior separation procedures is not possible due to the overlapping of their UV spectra and the presence of interfering excipients. The lactone ring of pilocarpine is known to be highly unstable in high temperature, and should be acylated using heptafluorobutyric anhydride. The trifluoroacetylated derivative was monitored using GC/MS. The analysis of pilocarpine by LC-UV is complicated, since it lack chromophoric group and is, thus, determined at low wavelengths (typically around 214 nm). The pre-column derivatization of pilocarpine with 4-bromomethyl-7-methoxycoumarin and subsequent LC with fluorescence detection, but the derivatization needed 12-48 h for completion. Capillary electrophoresis can be achieved high separation efficiencies and resolution and performed in different modes. However, it combined with UV absorbance detection, does not extract the more polar pilocarpine, the determination of which is essential for the investigation of metabolism of pilocarpine. Merbel et al [25] used LC-MS to determine pilocarpine but this method requires costly instrumenation. Due to the existence of the imidazole moiety enables pilocarpine to form complexes with divalent metal ions namely copper (II), cobalt (II), nickel (II) and zinc (II) so that this paper is concerned with a study of the chelation of pilocarpine with metal ions using votammetric techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) to develop of sensitive methods for the determination of pharmaceuticals (pilocarpine).

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研究方法

1.Preparation of chelate of pilocarpine

A 100 mg (0.408 m mole) amount of pilocarpine hydrochloride dissolved in aqueous sodium hydroxide and 5.30 mg (0.0408 m mole) metal chlorides dissolved in deionized water, then transferred both into a10 ml reaction flask and made up to10 ml volume with deionized water. This mixture was heated for 5, 10, 15, 20, 30, 40 and 50 min in a constant temperature water bath maintained at 50 ± 2℃.

3.Determination of the chelate of pilocarpine by voltammetry

CV and DPV experiments were performed using an EG&G Princeton Applied Research (Princeton, NJ) Model 394 connected to an EG&G325 Faraday cage with Smart stir and KO269 A Faraday cage. The *in situ* formulation of mercury –coated carbon fibre and gold wire microelectrodes, respectively, as working electrodes, were achieved according to our published paper [31]. Comparative tests of supporting electrolytes and pH such as Britton-Robinson buffer (pH 2.56-8.33), phosphate buffer (pH 2.12 and 6.60), acetate buffer (pH 4.50) and an aqueous containing 0.1 M tetrabutylammonium hydroxide (pH 12.01), lithium perchlorate (pH 6.01), sodium perchlorate, tetraethylammonium perchlorate, and tetraethylammonium tetrafluorobrate supporting electrolyte were conducted. A 1.0 g amount of ophthalmic solution and treatment of dry mouth (xerostomia) tablet sample was accurately weighed, dissolved in 10 ml of 0.1 M sodium bicarbonate aqueous and extracted three times with 6 ml chloroform. The aqueous phase and organic phase, respectively, were

collected, evaporated and transferred into a 10 ml calibrated flask and make up to a volume with methanol and deionized water (1:1 v/v).

. In order to obtain calibration graph for the chelation of pilocarpine, 10 ml of supporting electrolyte was pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min before votammetric measurement. By micropipette, aliquots of 40.8 mM pilocarpine solution were added. After each addition voltammograms were obtained; the solution de-aerated for 1min after each addition before obtaining the voltammogram. Quantitative analyses were performed in the differential pulse mode. The potential was set at –0.4 to –2.0 V *versus* Ag/AgCl reference electrode for reduction. The pulse height was 50 mV, and the scan rate 10 mVs^{-1} with a film electrode. For sample solution analysis, 1ml of the solution was pipetted to volume with 0.1 M lithum perchlorate solution. This solution was analysed by DPV using the same condition as for calibration graph.

3.Determination of chelate of pilocarpine by HPLC

Working standard solutions were prepared from a stock standard solution in methanol in the range 2- 40 mg/ml. RP- HPLC was performed on a Phenomenex Luna C_{18} 5mm (250 × 4.6 mm) column. The isocratic mobile phases were 10:90, 20:80, 30:70 and 40:60 *v/v*) methanol - phosphate buffer (pH 6.86) and methanol –lithium perchlorate (0.1-0.2 mM), respectively; the mobile phase flow rate was 0.3 ml/min. Construction of a flow-through polarographic detector combined with an ultraviolet detector (HPLC-UV) has been previously described [31] Detection after separation on the Phenomenex Luna C_{18} column was carried out using ultraviolet detector set at 235 nm. The polarographic detector was operated at –1.4 V. Using the injection value, 20 μl of the prepared sample solution and standard solution were chromatographed under the operating conditions described above. Quantitation was based on the peak area of the sample.

結果與討論

1.Choice of analytical method

To compare the electroanalytical utility of Hg/Au and Hg/GCE (glassy carbon electrode) modified electrodes, we studied the electrochemical experiments, in cyclic voltammograms (CV) on chelate of pilocarpine (Fig.1), and it was found that Hg/Au film gave a better performance than Hg /GCE, and was chosen for use in the determination of pilocarpine in pharmaceuticals. Cyclic voltammograms (CV) of the Co (II), Zn (II), Cu (II), and Ni (II) chelates with pilocarpine at the Hg/Au electrodes were shown in Fig. 2. Due to the Co (II) chelate was reduced to a less negative potential than the others that this complex was chosen to study the voltammetric behaviour, and to

determine the concentration of pilocarpine. CV of Co (II)-pilocarpine in Britton-Robinson buffer (5.01), phosphate buffer (pH 6.60) and lithium perchlorate (pH 6.01) solution with a mercury-modified gold electrode showed two well-defined reductions. Britton-Robinson buffered was peak current more higher than on the other, but peak potential more negative than the other. Therefore, the lithium perchlorate (pH 6.01) solution was used to Co (II)-pilocarpine determine levels in pharmaceuticals.

2. Voltammetric behaviors of Co (II)-pilocarpine

CV obtained using the standard (Co (II)-pilocarpine) addition method on an Hg/Au electrode (Fig. 3, the regression equations used was y = 37.78 x +14.73, the correlation coefficient was r =0.9990 for first wave –1.0 V; and y = 402.9 x + 49.28; r = 0.9985 for second wave – 1.40 V, respectively) showed two well-defined reduction peaks. In the reverse scan, no oxidation peak is observed (Fig. 3). The cathodic peak current i_p is found to increase with the scan rate (Fig.4) and the concentration of C_a (Fig. 3). Good linearity was observed between the peak height (current) and the square root of the scan rate ($v^{1/2}$ Fig.5). The regression equations used was y = 4.56 x - 9.08, the correlation coefficient was r =0.9905 for first wave –0.882 V; and y = 5.83 x-15.6; r = 0.9997 for second wave – 1.37 V, respectively). The i_p is proportional to C_a and $v^{1/2}$. The current function values (Ip = $i_p/AC_a v^{1/2}$) in lithium perchlorate and Britton-Robinson buffer (where A is area of electrode) were constant. This type of voltammetric behavior should correspond either to diffusion controlled irreversible charge transfer followed by an irreversible fast chemistry reaction [32]. Differential pulse voltammograms (DPV) obtained using the standard (Co (II)-pilocarpine) addition method on an Hg/Au electrode (Fig. 6, the regression equations used was y = 210 x +5.08 the correlation coefficient was r =0.9916 for first wave –0.69V; and y = 287 x +3.15; r = 0.9992 for second wave –1.20V, respectively) showed two well-defined reduction peaks. A representative DPV voltammogram of a commercial ophthalmic solution is shown in Fig.7

3. Separation of Co (II)-pilocarpine

Various ratios of methanol- –lithium perchlorate (0.1-0.2 mM) (10:9 0, 20:80, 30:70, 40:60 *v/v*) were experimented with on chelate of pilocarpine. After various studies of the retention behavior of the Co (II)-pilocarpine, baseline separation was achieved. Methanol-lithium perchlorate (0.2 mM) (30:70, *v/v*) was found to be the best mobile phase for a good resolution and for the least peak interference in the matrix. Fig.8 shows representative LC-EC and LC-UV chromatograms for the *Co (II)-pilocarpine.*

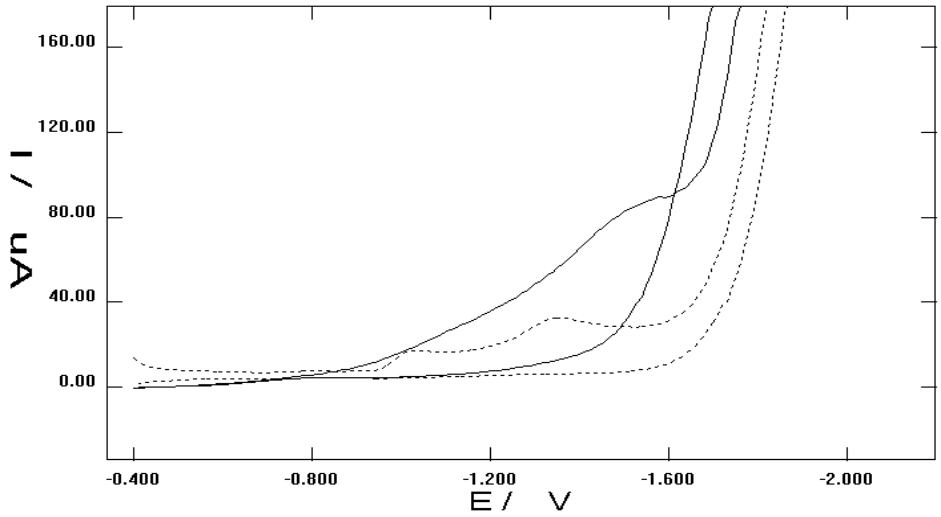

Fig.1 Cyclic voltammograms of pilocarpine (20.8 mM) and Co(0.130 mM) at different modified electrodes in 0.1 M LiClO4: (－)Hg (2 mM)/GCE and (----)Hg (2 mM)/Au, Scan rate 50 mV/s

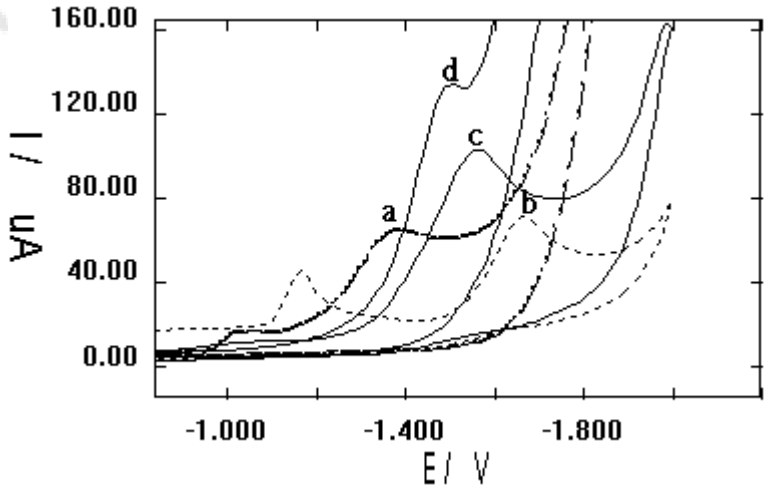

Fig. 2 Cyclic voltammograms of 4.17×10^{-2} M pilocarpine in 0.1 M $LiClO_4$ containing a variety of metal 1.35×10^{-4} M (a) Co (II);(b) Zn (II) (c)Cu(II); (d) Ni(II) , Scan rate50 mV/s.

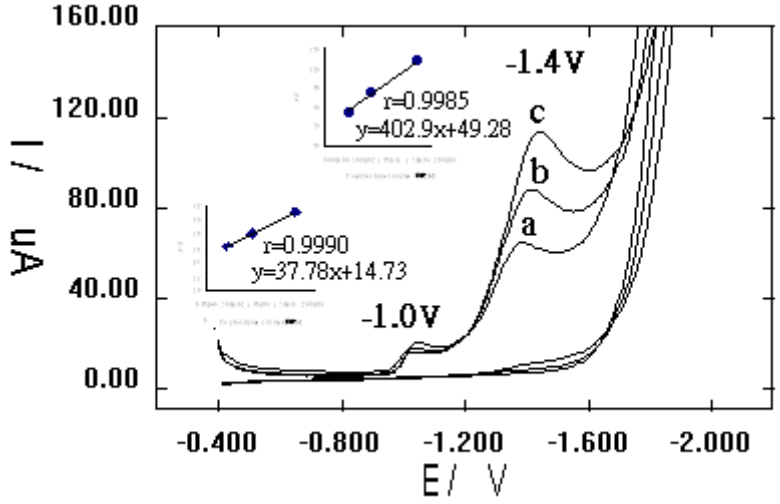

Fig. 3 Cyclic voltammograms of 0.1M $LiClO_4$ solution containing1.30x10^{-4} M cobalt and pilocarpine (a) 4.17x10^{-2} M; (b) 8.35 x10^{-2} M); (c) 16.7 x $^{-2}$ M. Scan rate 50 mVs^{-1}

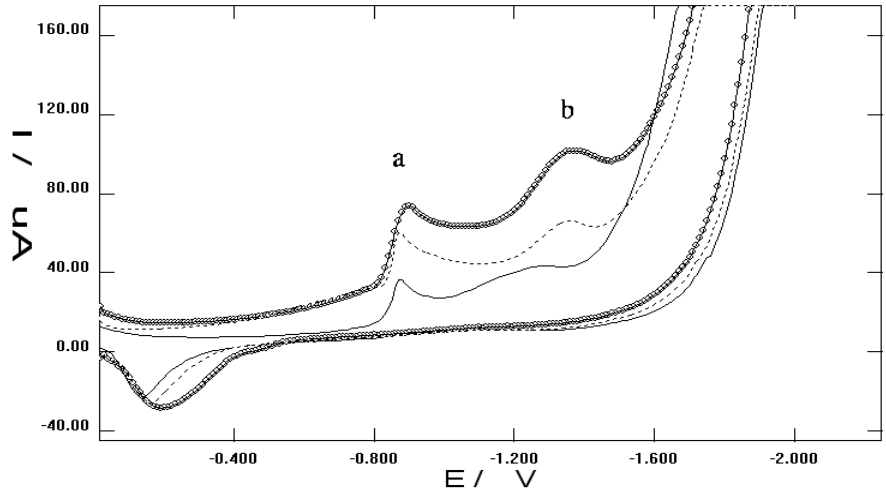

Fig.4 Cyclic voltammograms of 0.1M $LiClO_4$ solution containing Co (0.336 mM) and pilocarpine (3.27mM) on Hg (2 mM)/Au modified electrode at different scan rates (—) rate100 mV/s; (---) rate 200 mV/s;(-◊-◊-) rate 400 mV/s. Peaks: (a) first wave at -0.882 V; (b) second wave at-1.369 V.

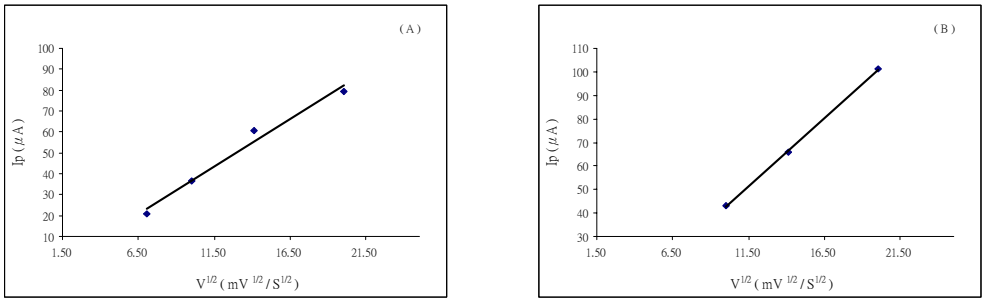

Fig. 5 Magnitude of the peak current, i_p, for Co-piloacrpine reduction as a function of square root of scan rate (A) first wave at -0.882 V; (B) second wave at-1.369 V.

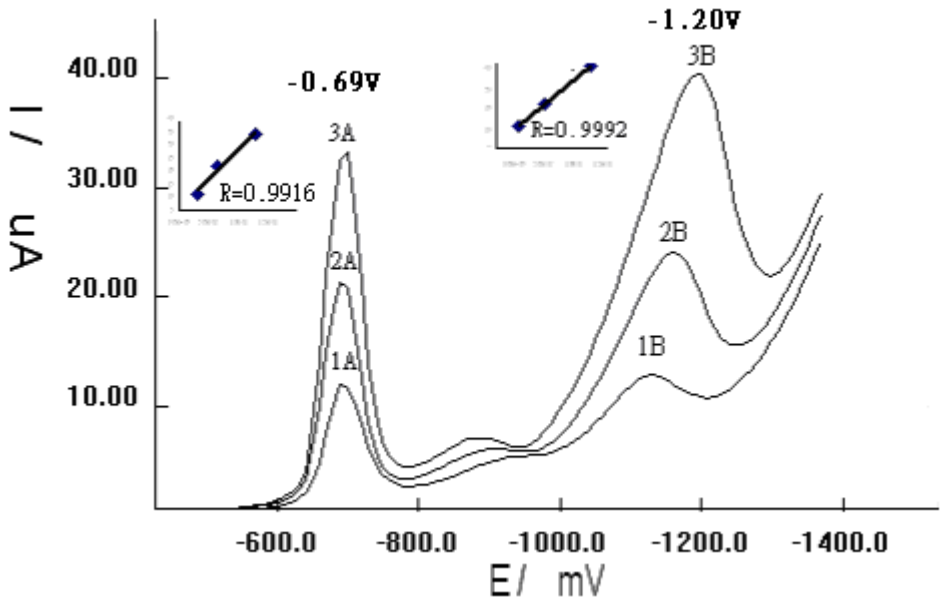

Fig.6 DPV obtained to produce calibration plot for Co- pilocarpine at a Hg (2mM) / Au .The current peak values were at –0.68 and –1.20 V (1) with 3.23 – 0.336 m mol of Co- pilocarpine, (2) with 6.53 –0.672 m mol of Co- pilocarpine, (3) with 13.1 –1.34 m mol of Co- pilocarpine. Scan rate 10 mV/s; pulse height 50.

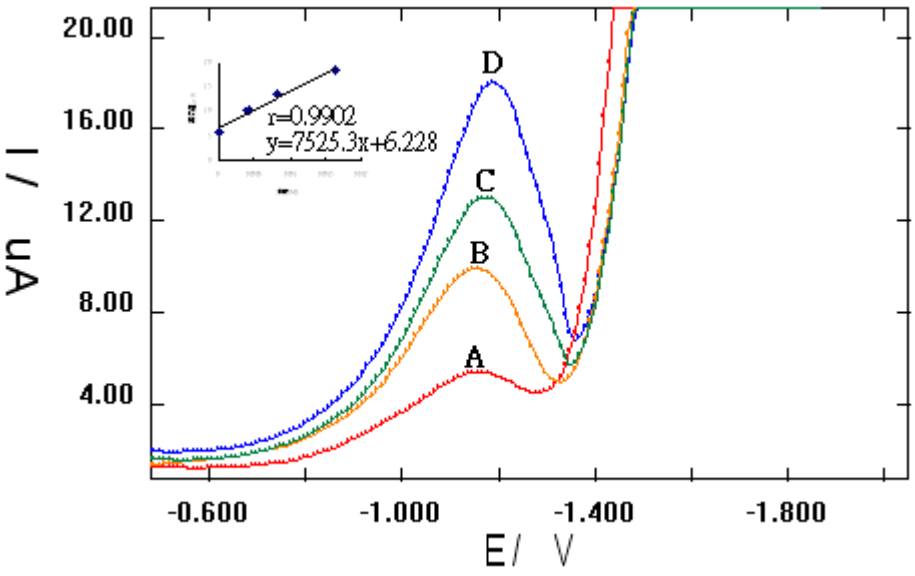

Fig. 7 Differential pulse voltammograms traces for pilocarpine of commercial ophthalmic solution at a Hg/Au. Peaks; A, -1.16 V, 5.44μ A (0 mM pilocarpine added); B, -1.16 V, 9.94 μ A (0.408 mM pilocarpine added); C, -1.18 V, 13.0 μ A (00.816 mM pilocarpine added); D, -1.19 V, 18.0 μ A (1.632 mM pilocarpine added); Scan rate 10 mV/s; pulse height, 0.05V.

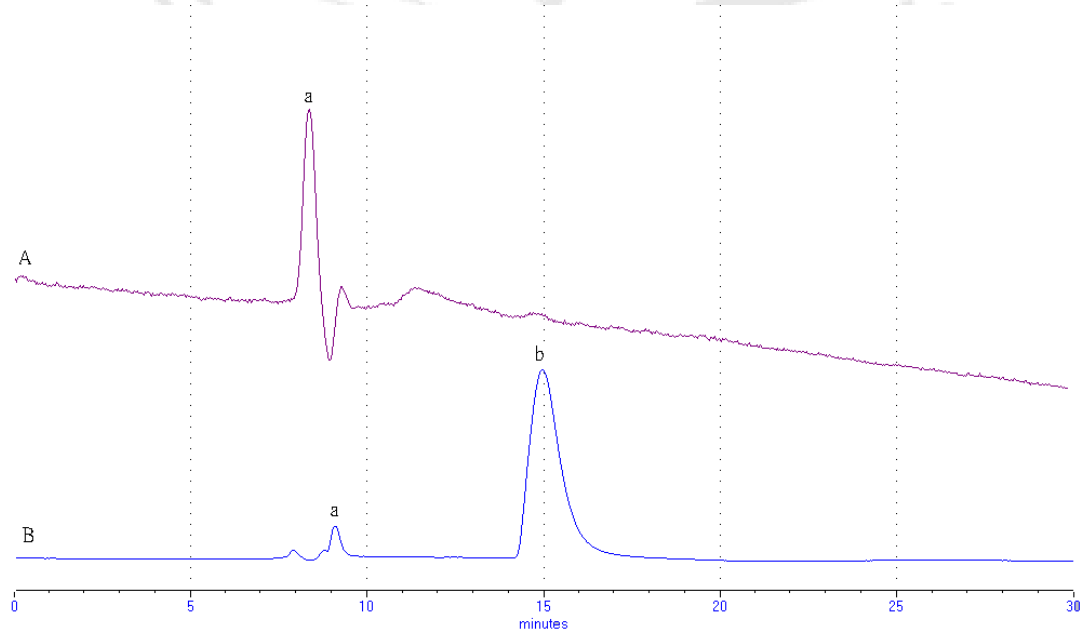

Fig. 8 Chromatograms obtained by (A) LC-ECD (B) LC-UV. Peaks: (a) Co-pilocarpine (b) pilocarpine.

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