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Automatic determination of amylocaine and bromhexine by atomic absorption spectrometry

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Abstract: An automatic method for the determination of amylocaine and bromhexine hydrochloride by atomic absorption spectrometry (AAS) is proposed. The drugs were determined indirectly by formation of reineckates, extraction into 1,2-dichloroethane and measurement of chromium in the organic phase. The chemical conditions and experimental variables influencing the performance of the flow system of the liquid–liquid extractor were established. The proposed method allows the determination of amylocaine and bromhexine at concentrations between 3 and 120 μg ml^{-1} with a relative standard deviation of 1 and 3%, respectively, even in the presence of other synthetic drugs.

Keywords: *Amylocaine; bromhexine; continuous liquid–liquid extraction; atomic absorption spectrometry.*

Introduction

Amylocaine hydrochloride (1-dimethylaminomethyl-1-methylpropyl benzoate hydrochloride) is a local anaesthetic of the ester type that causes reversible insensitization near the area where it is administered. Bromhexine hydrochloride (2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzylamine hydrochloride) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid mucus [1].

Amylocaine and bromhexine are usually determined by methods applicable to alkaloids that use chromatographic separation techniques such as TLC, GC and HPLC. One TLC system applicable to the separation and identification of 31 alkaloids and drugs was developed by Walash *et al.* [2] by comparing the absorbance ratios obtained from densitometric measurements of the developed plate with a multi-wavelength UV detector. This technique has also been used for separation of alkaloids after development with Dragendorff's reagent [3, 4]. Liquid chromatography has been used in conjunction with UV spectrophotometry [5] and mass spectrometry [6] for the extraction and determination of cinchona bark and cantharantus alkaloids, respectively. However, the chromatographic technique is laborious as it involves several time-consuming steps prior to injection; in addition, the more sensitive methods based on this technique usually entail preconcentrating the sample. These operations lead to decreased precision of results and to longer analysis times.

Alkaloids have also been determined by AAS methods involving extraction of ion-pairs using various metal-containing reagents. Thus, by using Reinecke's salt [7], $Co(SCN)_4^{2-}$ [8], Dragendorff's reagent [9] and $Ni(SCN)_4^{2-}$ [10], alkaloids can be determined at concentrations between 0 and 40 mg ml^{-1} by extraction into nitrobenzene or 1,2-dichloroethane. However, these methods are not highly sensitive and have the disadvantage that continuous introduction of organic solvents into the flame produces hazardous toxic gases. A continuous liquid–liquid extractor coupled on-line to an atomic absorption spectrometer has been used for the indirect AAS determination of active components in pharmaceuticals preparations [11, 12], with substantially higher sensitivity and/or selectivity than that afforded by manual methods and much safer as the volumes of toxic organic solvents inserted into the flame are typically only a few microlitres. A continuous liquid–liquid extraction system was recently used for the indirect AAS determination of cocaine using a variety of inorganic complexes such as BiI_4^-, $Co(SCN)_4^{2-}$, $Ni(SCN)_4^{2-}$, $Fe(SCN)_6^{3-}$ and Reinecke's salt to form and extract various ion-pairs with cocaine [13].

This paper reports the use of a simple liquid–liquid extractor coupled to an atomic ab-

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sorption spectrometer for the indirect determination of two synthetic medicinal bases (amylocaine and bromhexine) based on the classical Reinecke's salt, which forms ion-pairs with the two drugs that can be readily extracted into organic solvents. The proposed automatic method is a significant improvement on earlier manual methods in terms of reagent consumption and throughput.

Experimental

Apparatus

An atomic absorption spectrometer (Perkin–Elmer 380) furnished with a chromium hollow-cathode lamp was used and calibrated with an air–acetylene flame according to the manufacturer's recommendations. The spectrometer output was connected to a Radiometer REC-80 Servograph recorder. The flow system consisted of a Gilson Minipuls-2 peristaltic pump, a Tecator L-100-1 injection valve, an A-10T solvent segmentor and an A-4 T-shaped glass separator (Bifok) that accommodated an internal Teflon tube and displacement bottles for pumping 1,2-dichloroethane. Polyvinyl chloride pumping tubes and Teflon tubing for the coils were also used.

Reagents

Amylocaine hydrochloride and bromhexine hydrochloride were purchased from Jescuder (Spain) and Sigma Chemical Co. (USA), respectively. Stock solutions containing 1.000 g l^{-1} of either in distilled water and 1:20 v/v ethanol–water, respectively, were prepared and stored at 0–4°C in PVC containers prior to used. Aqueous solutions containing 10 g l^{-1} of the following basic salts were also used: sparteine sulphate, atropine sulphate, pilocarpine hydrochloride, cocaine hydrochloride, procaine hydrochloride, ephedrine hydrochloride, lidocaine hydrochloride, papaverine hydrochloride. Codeine and strychnine were dissolved in 1:3 v/v ethanol–water and ethanol, respectively. Reinecke's salt (ammonium tetrathio-cyanodiammonochromate) was purchased from Sigma Chemical Co. (St Louis, MO, USA) and dissolved in distilled water at a concentration of 1% w/v.

General procedure

The manifold used for the formation of reineckates and their simultaneous extraction

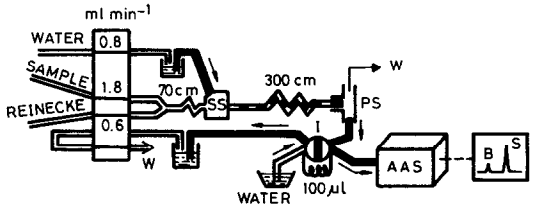

Figure 1
Scheme of the continuous liquid–liquid extraction system used for the indirect determination of amylocaine and bromhexine. SS, solvent segmentor; PS, phase separator; I, injector; W, waste.

is depicted in Fig. 1. First, a sample containing 3–80 or 5–120 µg ml^{-1} of amylocaine or bromhexine hydrochloride at pH 2.0 or 1.5 was continuously pumped into the system and mixed with the 0.1 or 0.05% Reinecke's salt carrier solution (for amylocaine and bromhexine, respectively) at pH 5. Reineckates were formed in a 70-cm long coil and subsequently extracted into 1,2-dichloroethane from a displacement bottle. A fraction of the organic extract (100 µl) was injected via injector I into a water stream (4 ml min^{-1}) that was directly aspirated by the nebulizer. The chromium present in the reineckate was then measured. A blank measurement was also made in parallel. The difference between the signals (sample and blank) was proportional to the drug concentrations in the sample. Calibration curves were obtained by linear regression analysis.

Results and Discussion

Reinecke's salt is a classical reagent in pharmaceutical analysis. Several colorimetric methods for the identification of alkaloids and their salts in pharmaceutical preparations use ammonium reineckate solutions in acetone as reagent [14]. This compound has also been used for the indirect AAS determination of various alkaloids [7] with no interference from 15 common inorganic ions, glutamate, starch or sucrose.

Effect of pH and the concentration of Reinecke's salt

The variables influencing the system performance were optimized by the univariate method. Several experiments were carried out in order to determine the effect of pH on the formation of the reineckates. First, the influence of the sample pH (30 or 50 µg ml^{-1} of

amylocaine or bromhexine hydrochloride, respectively) was investigated over the range 0.3–5.0. As can be seen in Fig. 2 reineckates were only formed below pH 3.8. Above pH 4, the reineckates precipitated, so the extraction efficiency was diminished as a result. The selected pH values for the amylocaine and bromhexine hydrochloride solutions were 2.0 and 1.5, respectively. A pH 0.3–6.0 for the carrier solution (0.25% Reinecke's salt) was found to be appropriate for the determination of both drugs, so, for simplicity, the pH obtained on diluting Reinecke's salt in water (*ca* 5 was chosen).

The optimum concentration of Reinecke's salt (carrier solution) was also determined by assaying concentrations between 0.01 and 1% (w/v). Figure 3 shows the influence of such a concentration on the determination of both drugs; that of 0.05 and 0.1% was chosen for the determination of bromhexine and amylocaine

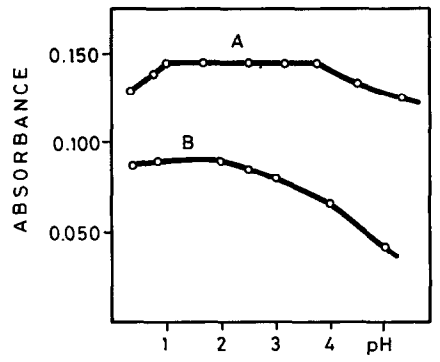

Figure 2
Effect of the pH of the amylocaine (A) and (B) bromhexine solution on absorbance. The drug HCl concentration was 50 μg ml^{-1} in both cases.

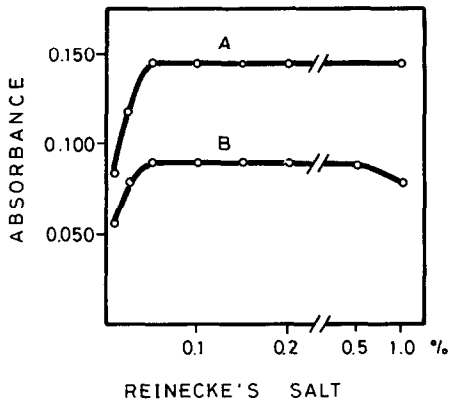

Figure 3
Influence of the concentration of Reinecke's salt on the determination of 50 μg ml^{-1} amylocaine (A) or bromhexine (B) HCls.

hydrochloride, respectively. Under these conditions, the absorbance of the blank ranged from 0.025 to 0.035, so a blank extraction was required in all instances.

Optimization of the flow system

In previous work [13] it was found that a membrane phase separator (with Fluoropore membranes) was inappropriate for this type of application because membranes were rapidly deteriorated by the solvent (1,2-dichloroethane), so a T-shaped glass-Teflon separator was used instead. With this T-shaped separator, an aqueous–organic phase flow rate ratio higher than 3 had a substantially adverse effect on performance since it gave rise to irreproducible measurements as a result of the aqueous phase reaching the flame. A sample flow rate of 1.8 ml min^{-1} and an organic phase flow rate of 0.8 ml min^{-1} were thus chosen for all ion-pairs assayed, taking into account the mutual influence of reproducibility, concentration ratio and sampling frequency. The carrier flow rate was kept at 0.33 times the sample flow rate in order to avoid excessive dilution of the sample. Tube lengths greater than 70 cm (0.5 mm i.d.) between the point of mixing of the drugs and carrier stream and the solvent segmenter (ion-pair reaction coil) had no effect on the absorbance. At smaller reactor lengths, the sample–reagent residence time was too short, so formation of the ion-pairs was incomplete. The extraction coil length was varied from 100 to 500 cm. A plot of signal versus extraction coil length showed the absorbance to increase with increasing length up to 160 and 220 cm for amylocaine and bromhexine, respectively. The signal remained constant at greater lengths up to 500 cm. An extraction coil length of 300 cm (0.5 mm i.d.) was finally chosen for the determination of both drugs. The extracted sample volume that was injected into the water line had a significant effect on the absorbance. Thus, the signal increased with increasing injected sample volume up to 80 and 100 μl for amylocaine and bromhexine, respectively. Somewhat lower volumes (100 μl) were chosen in both instances in order to avoid the toxic hazards of vapours (particularly those of HCl and phosgene).

Figures of merit

The effect of the concentration of amylocaine and bromhexine hydrochlorides on the absorbance was studied by measuring the peak

height yielded by different solutions that were processed through the manifold depicted in Fig. 1. The regression equations obtained were as follows:

$$A = 0.002 + 2.9 \times 10^{-3}x \ (r = 0.9997);$$
$$A = 0.001 + 1.75 \times 10^{-3}y \ (r = 0.9998),$$

where x and y denote the concentration of amylocaine and bromhexine hydrochlorides (in $\mu g\ ml^{-1}$), respectively; A is the absorbance, and r the correlation coefficient. The proposed method permits the determination of amylocaine and bromhexine hydrochlorides over the concentration ranges 3–80 and 5–120 $\mu g\ ml^{-1}$, respectively. The detection limits were calculated as three times the standard deviations of the absorbances obtained in 30 injections of the blanks and proved to be 2.1 and 2.8 $\mu g\ ml^{-1}$ for amylocaine and bromhexine HCls respectively. The precision was determined by injecting 11 samples containing 30 or 50 $\mu g\ ml^{-1}$ amylocaine or bromhexine HCls, respectively; the relative standard deviations obtained were 3.4 and 1.0%, respectively.

Because Reinecke's salt is a classical reagent for identification of basic analytes, the effect of a number of common synthetic medicinal bases which can also form reineckates was investigated. Table 1 lists the tolerated limits for potentially interfering drugs in the determination of amylocaine and bromhexine hydrochlorides by the proposed method. Ephedrine was the only drug tolerated at concentrations at least 100-times higher than those of amylocaine and bromhexine. Cocaine interfered at concentrations similar to those of amylocaine and bromhexine because, as stated elsewhere [13], the same system permits its determination with Reinecke's salt.

Table 1
Tolerated limits* of foreign drugs in the determination of 30 $\mu g\ ml^{-1}$ amylocaine or 50 $\mu g\ ml^{-1}$ bromhexine (as hydrochlorides) by the Reinecke method

Alkaloid or base	Amylocaine	Bromhexine
ephedrine	>100	>100
sparteine	25	15
codeine	15	15
pilocarpine	15	15
procaine	15	10
strychnine	8	5
atropine	3	3
lidocaine	3	2
papaverine	2	2
cocaine	<1	<1
amylocaine	—	<1
bromhexine	<1	—

*Ratio of foreign synthetic drug to analyte concentration.

Conclusions

The earlier manual method [7] involves tedious sample manipulation and provides a linear determination range for bases (strychnine, quinine, emetine, tetracaine, procaine and ephedrine) which falls between 1.5 and 100 $\mu g\ ml^{-1}$, similar to those of the proposed method for amylocaine and bromhexine. However, the organic extractant used in the manual method, nitrobenzene, is hazardous when continuously inserted into the flame and has an unpleasant smell; by using a continuous liquid–liquid extractor, the organic solvent used in the proposed method is held in closed displacement bottles, so only a few microlitres are introduced into the flame. The proposed flow injection method takes *ca* 2 min per analysis compared to over 30 min in the manual procedure (20 min for phase separation alone). In addition to increased throughput, it affords reproducible results that can be obtained by unskilled personnel and uses reagents more sparingly.

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