Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 2, pp. 179–184, 1994

0731-7085/94 $6.00 + 0.00

Determination of papaverine and cocaine by use of a precipitation system coupled on-line to an atomic absorption spectrometer

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Abstract: A continuous-precipitation flame-atomization atomic absorption spectrometric method for the determination of papaverine and cocaine hydrochlorides is proposed. The method is based on the precipitation of reineckates by injection of Reinecke's salt into a carrier containing the alkaloids and their subsequent retention on a stainless steel filter. In this way, papaverine and cocaine hydrochlorides can be determined over the ranges 5–85 and 50–850 μg ml^{-1} with a relative standard deviation of 1.3 and 3.2%, respectively, and a sampling frequency of 150 h^{-1}. The proposed method is more sensitive and selective for papaverine than it is for cocaine and can be applied to the determination of papaverine HCl in pharmaceutical preparations.

Keywords: *Papaverine; cocaine; continuous precipitation; Reinecke's salt; atomic absorption spectrometry.*

Introduction

Papaverine (6,7-dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline) is an alkaloid that can either be obtained from opium or prepared synthetically; it has an antispasmodic action that inhibits phosphodiesterase and relaxes smooth muscle directly. It is also occasionally given to relieve ischaemia and the symptoms of senile dementia. Cocaine (methyl benzoylecgonine) is another alkaloid, which is extracted from cocoa leaves or synthesized from ecgonine. It is normally used only as a surface anaesthetic for the eye, ear, nose and throat because of its potential systemic toxic effects when administered by other routes. This alkaloid is a psychotropic drug with a long history of human consumption [1].

Owing to the similarities in the chemical properties of the alkaloids, papaverine and cocaine are usually determined by methods based on chromatographic separation techniques such as TLC [2] or HPLC, using a photodiode array [3] or MS detector [4]. Opium alkaloids can be determined by TLC combined with flame ionization detection (the calibration graph for papaverine is linear from 1 to 7 μg [5]). These alkaloids have also been determined in poppy straw by HPLC with detection at 254 nm [6]; the method used for this purpose involves several manual operations including extraction, filtration and concentration prior to injection onto a column of μBondapak CN; recoveries obtained in this way range from 95 to 100%. Papaverine has also been determined in pharmaceutical preparations by UV spectrophotometry at 250 nm. The enantiomeric composition of cocaine samples was established by HPLC with UV detection [8]; also, whole blood enriched with cocaine to 9 μg ml^{-1} was measured by GC [9] for degradation studies during procurements, transport and short-term storage. Robotic extraction of cocaine and benzoylecgonine from blood by solid-phase chemistry was used prior to derivatization by trimethylsilation followed by GC–MS analysis [10]. Alcohol-specific cocaine metabolites in serum and urine have also been determined over the range 25 to 5000 ng ml^{-1} [11], and qualitatively assayed with the Roche ONTRAK kit for screening of drugs of abuse [12] with results consistent with those obtained by RIA with GC–MS confirmation.

The formation of ion pairs between alkaloids and drugs with $Co(SCN)_4^{2-}$ [13], $Ni(SCN)_4^{2-}$ [14] and Dragendorff's reagent [15] followed by extraction of the ionic complex and direct nebulization in the flame of an atomic absorption spectrometer have been used for the direct

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determination of these organic compounds in the range 0–40 mg ml^{-1}. Reinecke's salt has also been used for the indirect determination of alkaloids over the range 1.5–100 μg ml^{-1} by atomic absorption spectrometry (AAS) [16] with a sampling frequency of 30 min per analysis. As a rule, these methods are not very sensitive and require intensive manipulation, which results in low sampling frequencies and irreproducibility of results. From this standpoint, flow injection is a major alternative to manual procedures, especially when separation techniques are involved. A continuous liquid–liquid extraction system was recently used for the indirect AAS determination of cocaine [17] or amylocaine and bromhexine [18] using Reinecke's salt. Also, a continuous precipitation system coupled to an AAS detector was developed for the determination of sulphonamides and local anaesthetics in pharmaceutical preparations and urine [19] by precipitation with copper and cobalt, respectively, over the range 2–35 μg ml^{-1}, with sampling frequency of 100–150 h^{-1}.

In this work, the formation of reineckates with the two alkaloids was automated by using a reversed-flow injection system. The resulting method is very simple and rapid, and permits the determination of papaverine.HCl and cocaine.HCl over the ranges 5–85 and 50–850 μg ml^{-1}, respectively. It was applied to the determination of papaverine hydrochloride in pharmaceutical preparations.

Experimental

Apparatus

A Perkin–Elmer 380 atomic absorption spectrometer equipped with a bead impact system in the burner chamber was used. A chromium hollow cathode lamp, operated at 25 mA, was also used at a wavelength of 357.9 nm. An acetylene flow rate of 3.4 l min^{-1} and an air flow rate of 19.5 l min^{-1} were employed to obtain a lean flame. The spectrometer output was connected to a Radiometer REC-80 Servograph recorder. The flow system comprised a Gilson Minipuls-2 peristaltic pump, a Rheodyne 5041 injection valve, a Rheodyne 5301 switching valve and a Scientific System 0.5–105 column furnished with a removable screen-type stainless steel filter (pore size 0.5 μm; chamber inner volume 580 μl; filtration area, 3 cm^2). Poly(vinyl choride) pumping tubes and PTFE tubing (0.5 mm i.d.) for the coils were also used.

Reagents

Papaverine hydrochloride and cocaine hydrochloride were purchased from Sigma (St Louis, MO, USA). Stock solutions containing 1,000 g l^{-1} of either in distilled water were prepared and stored at 0–4°C in PVC containers prior to use. Aqueous solutions containing 10 g l^{-1} of the following basic salts were also used: sparteine sulphate, atropine sulphate, pilocarpine hydrochloride, amylocaine hydrochloride, procaine hydrochloride, ephedrine hydrochloride and lidocaine hydrochloride. Strychnine, bromhexine hydrochloride and codeine were dissolved in ethanol and ethanol–water (1:3, v/v), respectively. Reinecke's salt (ammonium tetrathiocyanodiammonochromate) was purchased from Sigma and dissolved in distilled water at a concentration of 1% w/v.

Samples

The contents of one ampoule (injectables), a volume of 0.8 ml of oral drops or 20 ml of syrup was dissolved in 250 ml of 100 ml of distilled water. For tablet formulations, five tablets of each sample were placed in a mortar and ground to a fine mesh; a portion of *ca* 0.4 g (Sulmetin) or 3 g (Salvacolina) of the resulting powder was accurately weighed and the powder was transferred into a 100 ml vessel and diluted to 50 ml with water, after which the solution was shaken mechanically for 20 min and filtered; the filtrate was transferred into a 250 ml (Sulmetin) or 100 ml (Salvacolina) volumetric flask and made to the mark with water. For continuous-flow analyses, aliquots of 5–6 ml of these solutions were placed in 25 ml volumetric flasks and diluted to the mark with water.

General procedure

The reversed-flow injection manifold used for the continuous precipitation of reineckates, which included a selecting valve to make blank measurements, is shown in Fig. 1. First, the precipitating reagent, 0.2% (papaverine) or 0.25% w/v Reinecke's salt (cocaine) at pH 5.0 was injected into a stream of 10^{-2} M HCl (blank) and a high peak due to chromium is obtained as a result. The selecting valve is then switched and the alkaloid sample (5–85 μg ml^{-1} papaverine.HCl or 50–850 μg ml^{-1}

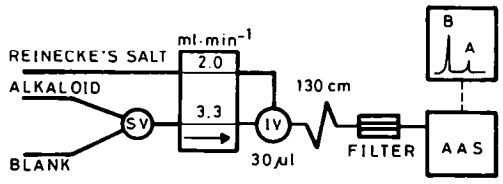

Figure 1
Scheme of the continuous precipitation system used for the indirect determination of papaverine and cocaine. IV, injection valve and SV, switching valve.

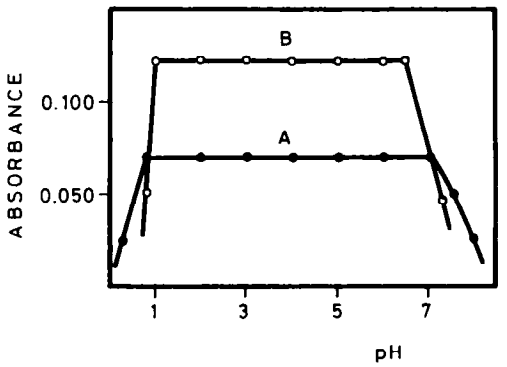

Figure 2
Influence of pH on the determination of 20 µg ml^{-1} papaverine.HCl (A) and 500 µg ml^{-1} cocaine.HCl (B). The pH of the precipitating solution, 0.2% Reinecke's salt, was kept constant at 5.0.

cocaine.HCl) in 10^{-2} M HCl was continuously pumped into the system; another identical injection of Reinecke's salt caused the alkaloid to form a precipitate which was retained on the filter; a low peak was thus obtained which decreased with increasing alkaloid concentration in the sample. The difference between the two peaks provided the amount of precipitated chromium, which was proportional to the alkaloid concentration in the sample. The filter was washed daily with 2 M ammonia in an ultrasonic bath for 3 min. All reagents and instruments were kept at room temperature throughout the experiments.

Results and Discussion

Reinecke's salt is a classical reagent for the identification of alkaloids and synthetic medicinal bases in pharmaceutical preparations [20]; it has also been used for the indirect AAS determination of various bases by manual [16] and automatic [17, 18] extraction into nitrobenzene and 1,2-dichloroethane, respectively, with advantages for the automatic procedure.

Chemical variables

The variables influencing the performance of the system were optimized by the univariate method. The alkaloid concentrations used for this purpose were 20 µg ml^{-1} papaverine.HCl and 500 µg ml^{-1} cocaine.HCl. The pH of the alkaloid solution and blank (water) was adjusted between 0.3 and 8.0 with diluted HCl or ammonia. As can be seen in Fig. 2, the reineckates were formed over a wide pH range; an acid medium was chosen in order to enhance the selectivity to avoid the interference of other basic drugs that can also precipitate as reineckates. For this purpose, a pH of 2.0 was selected which was readily obtained by preparing the samples in 10^{-2} M HCl. A pH between 0.3 and 9.2 for the precipitating reagent (0.2% Reinecke's salt) was found to be appropriate for the determination of both drugs, so, for simplicity, the pH obtained on diluting Reinecke' salt in water (*ca* 5.0) was adopted.

The effect of Reinecke's salt concentrations between 0.05 and 0.3% w/v on the analytical response was studied at a constant injected volume of 30 µl. The sensitivity to papaverine was found not to be affected over the interval 0.05 to 0.25%; on the other hand, no steady state was reached in the determination of cocaine, obviously owing to an inadequate reagent concentration for complete precipitation of 500 µg ml^{-1} cocaine.HCl. Reinecke's salt concentrations above 0.25% w/v pushed the blank signal outside the linear range of the instrument for chromium measurements, so a water stream had to be included for dilution of the reagent cation prior to the nebulizer. However, the diluting stream resulted in dramatically decreased differences between the blank and sample signals and also decreased precision in the results. Therefore, a 0.2 and 0.25% Reinecke's salt concentration was used for the determination of papaverine and cocaine, respectively, bearing in mind that, even though cocaine reacted incompletely, the results obtained were still reproducible as typical for a continuous precipitation method.

The influence of temperature on the precipitation reaction was studied over the range 20–85°C by thermostating the sample, reagent solutions, precipitation coil and filter. The signal was not affected up to 40°C, above which it started to decrease probably through sample

decomposition. Therefore, it was advisable to work at room temperature.

Flow injection variables

The optimized flow injection parameters included the sample flow rate, injected volume of Reinecke's salt and dimensions of the precipitation reactor. The sample flow rate was varied between 1.2 and 4.5 ml min^{-1}. This variable was quite significant: low flow rates resulted in wider, lower peaks; the absorbance difference between blanks and samples increased up to 2.6 ml min^{-1}, and remained constant at higher alkaloid flow rates. A sample flow rate of 3.3 ml min^{-1} was thus selected because higher flow rates gave rise to increased blank signals and irreproducibility in the results.

The influence of the volume of Reinecke's salt on the output was studied between 30 and 120 μl. A preliminary study of the influence of Reinecke's salt showed the maximum acceptable concentration of this reagent for an injected volume of 30 μl to be 0.25% — higher contents pushed the blank signal beyond the instrument's linear range for chromium. Therefore, in order to study the effect of injected volumes above 30 μl, the Reinecke's salt content was decreased to 0.1% for the determination of both papaverine and cocaine.

As shown in Fig. 3, no steady state was reached in the determination of cocaine because increased injected volumes of Reinecke's salt had the same effect as increased reagent concentrations within the reaction plug. Therefore, there were two choices for the cocaine determination: using 70 μl of 0.1% Reinecke's salt or 30 >l of 0.25% reagent. We selected an injected volume of 30 μl for the determination of papaverine (0.2% Reinecke's salt) and cocaine (0.25% Reinecke's salt) because of the higher precision achieved in the blank signals at lower injected volumes.

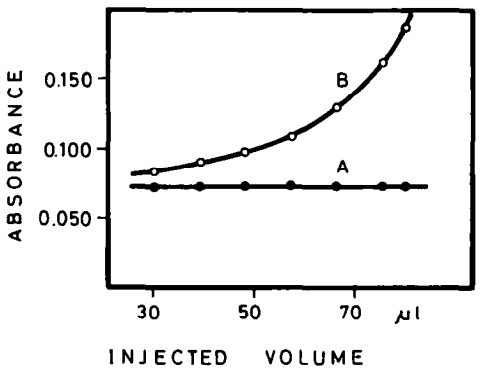

Figure 3
Effect of the injected volume of 0.1% Reinecke's salt on the determination of the alkaloids. Alkaloid concentrations as in Fig. 2.

The precipitation of reineckates was a fast reaction and no differences were observed in the peak heights when the reaction coil length was varied between 10 and 300 cm (0.5 mm i.d.). A coil 130 cm long and 0.5 mm i.d. was therefore chosen, which resulted in a residence time of 5 s, to precipitate each reineckate.

Analytical application

The proposed reversed-flow injection assembly provided linear calibration graphs over the papaverine.HCl and cocaine.HCl concentration rantes 5–85 and 50–850 μg ml^{-1}, respectively. The equations of the calibration curves were as follows:

$$A = -3.7 \times 10^{-4} + 3.4 \times 10^{-3} \text{ (papaverine.HCl)} \quad (r = 0.999),$$

$$A = -2.1 \times 10^{-3} + 3.3 \times 10^{-4} \text{ (cocaine.HCl)} \quad (r = 0.998),$$

where A is the peak height difference and concentrations are expressed in μg ml^{-1}. The detection limit, 2 (papaverine) and 25 μg ml^{-1} (cocaine), was calculated as three times the standard deviation of the peak height for 30 injections of a 10^{-2} M HCl solution (blank). The precision of the method was checked on 11 samples containing 20 μg ml^{-1} papaverine.HCl or 400 μg ml^{-1} cocaine.HCl that were measured over a few days. The calculated relative standard deviation (RSD) was 1.3 and 3.2% (n = 11) for papaverine and cocaine, respectively. The sample throughput (calculated from the time required for each injection, allowing 5 s for the precipitation reaction and including processing of the blank) was *ca* 150 samples h^{-1}.

The tolerance of the method potential interferences from common synthetic medicinal bases that can also form reineckates was investigated. Table 1 lists the tolerated limits for potentially interfering drugs in the determination of papaverine and cocaine hydrochlorides by the proposed precipitation method. Foreign synthetic drugs were added at a maximum level of 2 mg ml^{-1} or 4 mg ml^{-1} per 20 μg ml^{-1} or 400 μg ml^{-1} papaverine or cocaine hydrochloride (tolerated ratio, 100:1 or 10:1), respectively.

Table 1
Tolerated limits* of foreign drugs in the determination of 20 μg ml^{-1} papaverine or 400 μg ml^{-1} cocaine (as hydrochlorides) by continuous precipitation with Reinecke's salt

Alkaloid or base	Papaverine	Cocaine
Sparteine	40	>10
Pilocarpine	30	>10
Codeine	10	5
Procaine	10	5
Lidocaine	10	5
Atropine	10	5
Amylocaine	10	4
Ephedrine	5	2
Strychnine	5	2
Bromhexine	5	2
Papaverine	—	<1
Cocaine	2	—

*Ratio of foreign synthetic drug to analyte concentration.

The proposed method was applied to the determination of papaverine.HCl in five commercially available pharmaceuticals. The results obtained in five individual determinations of papaverine ± standard deviation are shown in Table 2. Direct analyses of Salvacolina tablets and Histaverin syrup provided results that were inconsistent with the manufacturer's certified values (recoveries were lower than 80%), so the standard addition method was used to determine the alkaloid in these samples. For this purpose, 1.0 ml aliquots of dilute sample (from a 100 ml volumetric flask) dissolved as described under 'Experimental', were placed in 25 ml volumetric flasks and added variable concentrations of papaverine.HCl between 5 and 60 μg ml^{-1}. The results obtained for ampoules, tablets, oral drops and syrup were consistent with the contents stated by the manufacturers, which testifies to the high accuracy and precision of the proposed method.

Conclusions

Some interesting conclusions can be drawn by comparing the results provided by the proposed method. Thus, the method is more sensitive to papaverine than to cocaine; also, the method for allows both drugs to be assayed directly in the presence of other basic drugs, even in pharmaceutical preparations.

In previous work we developed a method for determining cocaine [17] and amylocaine and bromhexine [18] by continuous extraction of their reineckates into 1,2-dichloroethane. The proposed precipitation method has several differences from the extraction method. Thus, (a) the extraction method has a higher affinity for cocaine, amylocaine and bromhexine (with similar sensitivity) than for papaverine (the most sensitively determined by precipitation with Reinecke's salt); (b) in the extraction method, the steady state was reached in all determinations for a Reinecke's salt concentration of 0.025–0.050% w/v, whereas in the precipitation method the steady state was not reached for cocaine, which required higher concentrations of precipitating reagent owing to the lower sensitivity of the method; (c) the sensitivity of the extraction method to cocaine (expressed as the slope of the calibration graph) was 2.7×10^{-3} (range, 10–100 μg ml^{-1}), compared with 3.3×10^{-4} (range, 50–850 μg ml^{-1}) in the proposed precipitation method; (d) the selectivity is similar for both methods, yet in the extraction method the determination of cocaine was interfered by bromhexine, amylocaine and lidocaine versus papaverine only in the precipitation method; and (e) the sampling frequency was 5 times higher for the precipitation method than for the extraction method as a result of the greater simplicity of the precipitation flow system.

Table 2
Determination of papaverine hydrochloride in pharmaceutical preparations by continuous precipitation with Reinecke's salt

Sample	Nominal amount	Found (mg)
Sulmetin-papaverine*	30 mg per ampoule	31.2 ± 2.4
Sulmetin-papaverine	30 mg per tablet	28.5 ± 1.6
Salvacolina†	5 mg per tablet	4.8 ± 0.4
Espasmotropina, oral drops	20 mg per ml	19.6 ± 0.7
Histaverin, syrup†	80 mg per 100 ml	81.2 ± 4.2

*Intramuscular injectable.
†By use of the standard addition method.
$n = 5$.

Acknowledgement — The CICyT is acknowledged for financial support awarded (Grant No. PB90-0925).

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[*Received for review 14 June 1993; revised manuscript received 19 July 1993*]