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DETERMINATION OF QUININE IN BEVERAGES, PHARMACEUTICAL PREPARATIONS AND URINE BY ISOTACHOPHORESIS

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SUMMARY

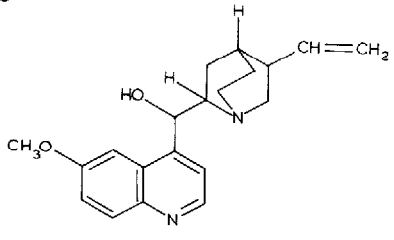
The suitability of isotachophoresis for the determination of quinine in different samples was investigated. The operational conditions were 0.01 *M* potassium–morpholinoethanesulphonic acid (MES) (pH 6.0) with 0.05% Mowiol as the leading electrolyte and *ca.* 0.005 *M* creatinine–MES as the terminating electrolyte. The analyses were carried out at 25 μA in a 0.2 mm I.D. PTFE capillary with UV and conductivity detection.

Quinine-containing beverages were degassed by sonification and directly injected. The limit of detection was 5 mg/l with a 4 μl injection volume. The allowed concentrations could be determined with sufficient accuracy. Analgesic preparations were dissolved in a solution of $5 \cdot 10^{-3}$ *M* MES with sonification. The quinine levels found agreed well with the declared values. The other constituents of the pharmaceuticals did not interfere with the analysis. Urine samples from volunteers were analysed after consumption of tonic. The samples were extracted with dichloromethane–isopropanol (95:5), vortexed, centrifuged, evaporated to dryness, the residue dissolved in $5 \cdot 10^{-3}$ *M* MES and analysed. At a concentration factor of 33, the limit of detection was *ca.* 60 μg in 48-h urine: 2–15% of the quinine consumed was excreted as the parent compound in the first 48 h after consumption.

The combination of the extraction procedure and the operational system makes the method suitable for the determination of a number of other alkaloids in physiological samples.

INTRODUCTION

Quinine [130-95-0] is a weak base ($pK_1 = 4.32$ and $pK_2 = 8.4$) with the following structure:



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It is widely used as an antimalarial drug, with a daily dose of *ca.* 1000 mg as 200-mg tablets. Some analgesic preparations also contain quinine at lower concentrations (< 50 mg per dose), resulting in a daily dose of 10–400 mg. Many of these preparations can be obtained without prescription. Soft drinks may contain varying amounts of quinine. According to Dutch Legislation on Consumer Goods (Warenwet), tonics should contain 40–85 mg/l and bitters up to 85 mg/l.

Because of the severe side-effects such as arrhythmias, hypotension, vomiting and certain neurological complications, the compound should not be used by children or by women during pregnancy and lactation. Only a few countries decree that soft drinks containing quinine must be labelled with these restrictions.

After oral intake, quinine is completely resorbed in the liver and the half-life in plasma is short (4–6 h). After 24 h the plasma concentration is negligible. It is mainly metabolized in the liver and excreted in the urine. Oxidation of both the quinoline and quinuclidine formed takes place, resulting in a series of phenolic and non-phenolic metabolites. In the human body it mainly metabolizes to (3*S*)-3-hydroxyquinidine, carbostyril 2'-quinidinone and O-desmethylquinidine[1–6], whereas 5–20% is not metabolized and is excreted in the urine[7,8].

In the past 5 years, a number of publications have appeared on the determination of quinine, 60% of these dealing with body fluids and 25% with beverages and pharmaceutical preparations. Chromatographic techniques have mostly been used: gas chromatography[9–11], gas chromatography–mass spectrometry[12,13], thin-layer chromatography[14,15] and high-performance liquid chromatography[16–23]. Spectrometry[24–26] has also been used.

This paper describes the determination of quinine in beverages, pharmaceutical preparations and human urine using capillary isotachophoresis.

EXPERIMENTAL

The isotachophoretic experiments were carried out in equipment developed and built by Everaerts *et al.*[27]. The separation compartment consisted of a PTFE capillary of length *ca.* 200 mm and I.D. 0.2 mm. The driving current was 25 μA, delivered by a modified high-voltage supply (Brandenburg, Thornton Heath, U.K.). UV absorption at 254 or 280 nm and a.c. conductivity were used for detection. The signals were recorded with a potentiometric recorder (Kipp, Delft, The Netherlands). Signal processing was achieved with an SP-4000 chromatographic integrator (Spectra Physics, Santa Clara, CA, U.S.A.) interfaced with a PUZZLE microprocessor (E. Steiner, Vienna, Austria). The operational system is listed in Table I.

All chemicals were of analytical-reagent grade purity (Merck, Darmstadt, F.R.G., Sigma, St. Louis, MO, U.S.A. or Fluka, Buchs, Switzerland). Deionized water was taken from a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). A stock solution of 30.3 mg/l quinine base (Fluka) in water was stable for 3 days when kept in the dark at 5°C.

The pharmaceutical preparations were purchased at a local drug store and the beverages at a local supermarket.

Sample preparation

Tonics and bitters were degassed by sonification and directly injected without filtration.

TABLE I

OPERATIONAL SYSTEM FOR THE DETERMINATION OF QUININE BY ISOTACHOPHORESIS

Driving current, 25 μA; 0.2 × 200 mm capillary; end-voltage, 10 kV.

Leading ion	Potassium
Concentration	0.01 *M*
Counter-ion	MES*
pH	6.0
Additive	0.05% Mowiol**
Terminating ion	Creatinine
Concentration	*ca.* 0.005 *M*
Counter-ion	MES*
pH	*ca.* 5.5

* MES = Morpholinoethanesulfonic acid (Sigma).
** Mowiol = poly(vinyl alcohol) (Hoechst).

Pharmaceutical preparations were ground with a mortar and dissolved in a solution of *ca.* $5 \cdot 10^{-3}$ *M* morpholinoethanesulphonic acid (MES) by ultrasonic treatment. The use of MES is favourable for both the solubility and the rate of dissolution. The time of sonification was 60 min.

For the urine samples, a 5-ml portion was adjusted to pH 11–12 with 400 μl of 2.5 *M* NaOH, extracted with 5 ml of dichloromethane–isopropanol (95:5), vortexed for 1.5 min and centrifuged for 45 min at 3000 *g*. A 3-ml volume of the organic phase was evaporated to dryness under N_2 and the residue dissolved in 100 μl of $5 \cdot 10^{-3}$ *M* MES solution by sonification for 30 min. The concentration factor thus obtained was 33.6.

An alternative extraction procedure for quinine in urine involves an adsorption on a C_{18} silica cartridge (Sep-Pak, Waters–Millipore, Milford, MA, U.S.A.). Here, 5 ml of urine plus 400 μl of 2.5 *M* NaOH was applied to the cartridge, which was washed with 5 ml of 10^{-3} *M* KOH. The quinine was eluted with 3 ml of methanol. The first 500 μl of the elute were discarded and the remaining 2.5 ml were evaporated to dryness under N_2 and the residue was dissolved in 100 μl of $5 \cdot 10^{-3}$ *M* MES solution by sonification.

RESULTS AND DISCUSSION

For an accurate determination of the zone length of quinine in the isotachopherogram, the UV signal was differentiated by the PUZZLE microprocessor, using a 12-bit analog-to-digital converter (ADC) at a sampling frequency of 20 s^{-1} and stored in memory. This differentiated signal was then sent to a chromatography integrator (SP-4000) by a 8-bit digital-to-analog convertor (DAC) at a rate of 7 s^{-1}. The inflection points in the UV trace were thus obtained from the "retention times" measured by the integrator. A similar method can be applied to the conductivity signal. The linearity of the method was good in the range 1–10 μg of quinine (the correlation coefficient was 0.9999).

A series of tonics and bitters purchased from different manufacturers were

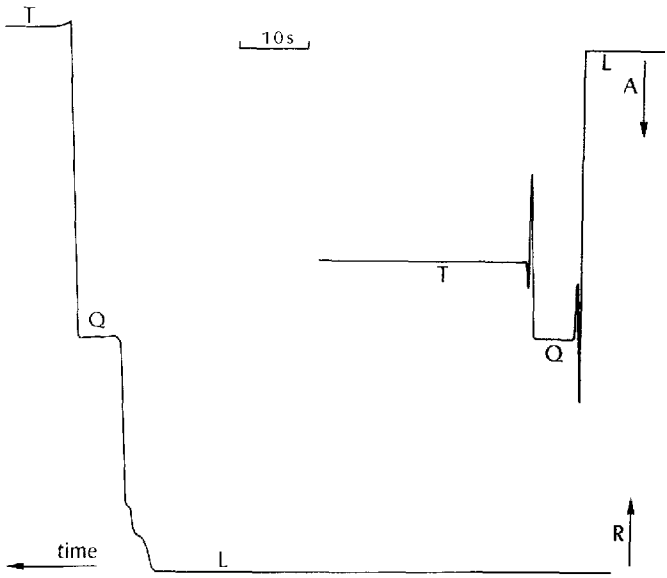

Fig. 1. Isotachophoretic analysis of quinine in a tonic. The zone length corresponds to a concentration of 43 mg/l. The conductivity signal (*R*) and the UV signal (*A*) at 254 nm are shown. The terminator (creatinine) also shows UV absorption.

analysed (Fig. 1). The amount injected was 4 μl and duplicate determinations were carried out. The results, summarized in Table II, indicate that the amounts allowed by the Dutch Legislation are well within the working range. The limit of detection of the method, without sample pre-concentration and injecting 4 μl, was *ca.* 5 mg/l.

The pharmaceuticals investigated were five analgesic preparations, obtainable without prescription. They contained quinine at varying concentrations in addition to a number of other constituents. The labelled composition is listed in Table III. The other constituents did not interfere in the analysis of quinine. Fig. 2 shows an isotachopherogram of the determination of quinine in an analgesic preparation. Care was taken that the sample was completely dissolved, the time of sonification being

TABLE II

QUININE CONTENT, CALCULATED AS FREE BASE, OF SOME BEVERAGES AS DETERMINED BY ISOTACHOPHORESIS

Sample	*Allowed** *(mg/l)*	*Found (mg/l)*
Bitter A	<85	30.2, 30.8
Bitter B	<85	30.2, 29.9
Bitter C	<85	37.6, 38.2
Tonic D	40–85	49.3, 48.2
Tonic E	40–85	52.0, 52.3
Tonic F	40–85	50.8, 48.7
Tonic G	40–85	39.0, 38.2

* According to Dutch Legislation on Consumer Goods.

TABLE III

QUININE CONTENT, CALCULATED AS FREE BASE, OF SOME ANALGESIC PREPARATIONS AS DETERMINED BY ISOTACHOPHORESIS

Sample	*Composition*	*Quinine content*	
		Labelled (mg)	*Found (mg)*
A	Quinine ethylcarbonate Antipyrine Phenacetin Caffeine	16.4	16.2, 16.1
B	Quinine citrate Aspirin Lithium citrate Starch	1.0	1.1, 1.0
C	Quinine sulphate Ascorbic acid	37.3	34.9, 34.3
D	Quinine sulphate Salicylamide Phenacetin Ascorbic acid Caffeine	20.8	20.8, 20.9
E	Quinine hydrobromide Thiamine hydrochloride	76.6	79.3, 77.7

varied from 2 to 60 min. The quinine concentration in the solution did not increase when sonification was prolonged to more than 40 min. The volume of solution injected was 4 μl and the quinine content found, determined in duplicate, correlated well with the declared value, calculated as the free base (see Table III). Also, the

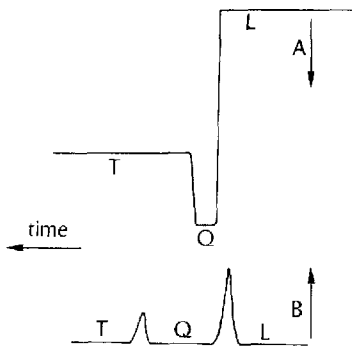

Fig. 2. Isotachopherogram of the analysis of a pharmaceutical dosage form containing 37 mg of quinine. The UV signal at 254 nm (A) is differentiated, stored in the computer memory and subsequently sent to the chromatographic integrator at reduced speed (B).

determination is sufficiently accurate to determine the stability of quinine in the dosage forms, *e.g.*, during storage.

Several workers[1–8] have reported on the metabolism of quinine. Although the half-life of quinine in plasma is short, preliminary isotachophoretic experiments have indicated[28] that between the 24th and the 48th hour after consumption 0–2% of the quinine is still excreted in the urine as the parent compound. For the determination of the total amount of quinine, excreted without metabolization, we therefore collected urine samples during 48 h after consumption instead of only during the first 24 h.

Other workers[29] have reported on the determination of quinine in blood by gas chromatography, following consumption of gin and tonic in a social setting. We worked under slightly modified conditions: for the determination of a blank, eight male volunteers, aged 23–42 years, weight 65–79 kg, refrained from consuming anything that might contain quinine for 1 week, then 48-h urine samples were analysed. The amounts found were <0.06 mg for each of the volunteers. In the actual experiments, the drinks were not mixed according to the so-called established methods[29]. Instead, each volunteer was given tonic in 100-ml amounts, which could be mixed with gin according to taste (addition of 0–40 ml of gin). No ice-cubes or lemon were added. All drinks were consumed within a period of 2 h. Collection of the urine samples was started immediately after the experiment and continued for the next 48 h. The samples were then stored in a freezer until use. Prior to analysis, the samples were treated according to the procedure described under Experimental. Initial experiments[28] indicated that both the solvent extraction and the cartridge adsorption procedures yielded good recovery of quinine added to a blank urine ($>95\%$). However, the solvent extraction, although slightly more time consuming, was clearly more

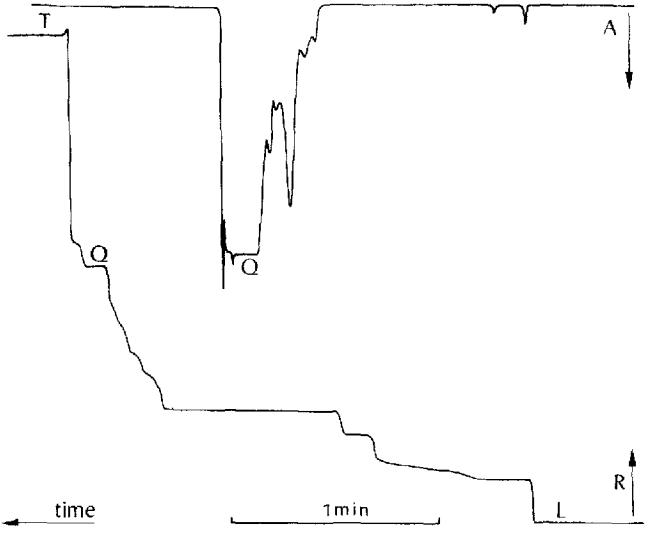

Fig. 3. Isotachopherogram of the analysis of quinine in an extract of human urine after consumption of tonic. The zone length corresponds to a concentration of 0.3 mg/l in urine. The conductivity signal (*R*) is shown together with the UV signal (*A*) at 280 nm. This wavelength allows a more specific detection of the quinine zone.

TABLE IV

URINARY EXCRETION OF QUININE, CALCULATED AS FREE BASE, AFTER CONSUMPTION OF TONIC, AS DETERMINED IN 48 h URINE BY ISOTACHOPHORESIS

The blank was <0.06 mg/48 h (see text).

Volunteer	*Intake (mg)*	*Urine/48 h (l)*	*Excreted*	
			mg	*%*
A	23.8	1.1	1.25	5.3
B	24.7	6.5	1.96	8.1
C	21.9	3.4	2.97	13.6
D	20.0	3.7	1.16	5.8
E	30.5	4.3	1.85	6.1
F	26.0	2.2	0.99	3.8
G	22.2	2.2	0.44	2.0
H	26.4	6.0	3.88	14.7

selective. This procedure was therefore chosen in order to avoid possible mixed zones in the isotachopherogram. Fig. 3 shows an example of the determination of quinine in urine after consumption of tonic and the results are summarized in Table IV. The amount consumed ranged between 20 and 30 mg. The quinine content of the tonic was determined for each of the 1000-ml bottles according to the method described. It can be seen from Table IV that the proportion of quinine that was excreted as the parent compound within 48 h ranged from 2.0 to 14.7%. These values agree with those cited in the literature.

CONCLUSIONS

Isotachophoresis provides a fast and reliable method for the determination of quinine in beverages and pharmaceutical preparations. The accuracy and precision enable stability studies, *e.g.*, of drugs during storage, to be carried out. The sample extraction procedure yields a concentration step that makes the technique suitable for the determination of quinine in physiological samples. Other alkaloids such as morphine and heroin can also be analysed with the present procedure, and the sensitivity of the method can easily be increased by modifying the concentration step of the extraction.

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