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Short Communications

Evaluation of the extraction of chloroquine from biological samples by different organic solvents

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Summary

A survey of the solvents used in the extraction of chloroquine during fluorimetric analysis of the drug was carried out. The solvents investigated were diethylether, chloroform, heptane, dichloromethane and dichloroethane. The percentage recoveries from all the organic solvents were reproducible between 1.00 and 0.05 μg/ml concentrations of chloroquine. Below 0.05 μg/ml, dichloromethane, dichloroethane and chloroform had poor reproducibility. Diethylether had the best reproducibility at 0.005 μg/ml concentration of chloroquine, and heptane had poor reproducibility below 0.01 μg/ml; diethylether was the best solvent for the extraction of chloroquine in biological fluids.

In the past, many analytical methods have been developed for the quantitative analysis of chloroquine in biological samples; the most common of these methods is fluorimetric analysis in which several solvents such as diethylether (Fournel, 1966), dichloromethane (Rubin et al., 1965) dichloroethane (Viala et al., 1973), heptane (Vogel and Konigk, 1975), and chloroform have been used as the extracting solvents for chloroquine. Although these solvents have been used at different stages for the extraction of chloroquine and its metabolites in biological samples, the efficiency of extraction of these solvents has not actually been compared. In order to get consistent results in the fluorimetric analysis of chloroquine, it is necessary to compare the efficiency of extraction of these solvents.

A fluorimetric assay which used diethylether was found to have a sensitivity limit of 5 ng/ml of chloroquine in biological samples (Adelusi and Salako, 1980). This method was found to be adequate for studying the disposition of chloroquine under different conditions. Since a sensitivity limit of 5 ng/ml of chloroquine was achieved in this method, the method has been re-examined in detail by using different organic solvents for the extraction of chloroquine during fluorimetric analysis. In so doing, the efficiency of extraction of different organic solvents was compared on the basis of their percentage recoveries of chloroquine in plasma.

Chloroquine sulphate (B.P.) was obtained from May&Baker, Dagenham, U.K. All solutions were made in distilled water and glassware were soaked

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in potassium dichromate solution overnight, washed and dried for 8 h at 100°C.

Venous blood was collected from healthy volunteers who had not taken chloroquine for one year into a heparin tube and immediately spun to separate the plasma from the red blood cells. The following reagents were also used; diethylether (Analar grade), heptane, chloroform, dichloromethane (BDH), 0.2 M NaOH in 50% (v/v) ethanol, borate buffer pH 7.85 and ammonia solution (spec. grav. 0.91).

Plasma containing chloroquine at concentrations 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 μg/ml were prepared and stored frozen at −10°C until analysed. The procedure for analysis was a modification of the method described by Adelusi and Salako (1980). 1 ml of the sample was placed in a 25-ml quickfit round-bottomed centrifuge tube. 0.2 ml of ammonia solution (spec. grav. 0.91) was added and extracted with 10 ml of diethylether (Analar grade) by shaking on a Gallenkamp electric shaker for 5 min. The aqueous and the organic layers were clarified by spinning in a centrifuge at 2500 rpm for 2 min. The organic supernatant layer was carefully pipetted into a clean tube and the extraction procedure repeated with a second 10-ml volume of ether. The ether extracts were bulked together and 16 ml of the organic layer was placed in a test-tube (quickfit) and 8 ml of phosphate borate buffer, pH 7.85, added and shaken for 2 min. 4 ml of the organic layer was partitioned into 8 ml of 0.1 M HCl by shaking for 2 min. The ether layer was aspirated off, 3 ml of the acid extract was mixed with 3 ml of 0.2 M NaOH in 50% (v/v) ethanol and 3 ml of phosphate borate buffer pH 7.85.

The fluorescence intensity of the solution was then measured on a Perkin-Elmer 204 Spectrofluorometer at excitation and emission wavelengths of 331 and 386 nm, respectively. The concentration of chloroquine in the samples was determined from a calibration curve and the percentage recoveries were then calculated. This procedure was repeated for heptane, dichloromethane, dichloroethane and chloroform with minor modifications for the last 3 solvents. Plasma without chloroquine was carried through the same extraction procedure and used as blank.

The percentages of chloroquine recovered from plasma for ether, heptane, dichloromethane, dichloroethane and chloroform are presented in Table 1. Ether had the highest percentage recovery while the recoveries from dichloromethane and dichloroethane were similar but lower when compared with those of ether, heptane and chloroform. The values for heptane and chloroform were lower than those for ether.

At concentrations between 1.0 and 0.05 μg/ml, all the solvents gave reproducible results but below 0.05 μg/ml, dichloromethane, dichloroethane and chloroform showed poor reproducibility. Heptane showed poor reproducibility below 0.01 μg/ml and only ether had good reproducibility at 0.005 μg/ml.

During the fluorimetric reading, high blank readings were obtained for heptane, dichloromethane, dichloroethane and chloroform. These high blank readings might be responsible for the large standard deviations obtained with these solvents compared with ether. The poor reproducibility below 0.05 μg/ml for dichloromethane, dichloroethane and chloroform and 0.01 μg/ml for heptane, may also be accounted for in this way.

The dielectric constants of the solvents considered in this investigation are heptane 1.0, diethylether 4.3, dichloromethane 9.1, dichloroethane 9.2, and chloroform 5.0 all at 20°C (Fieser and Fieser, 1976). Diethylether has a dielectric constant intermediate between that of heptane and dichloromethane or dichloroethane. It is thus less selective than heptane but more selective than dichloroethane or dichloromethane. Dichloromethane and dichloroethane are the most polar of all the 5 solvents considered; this polarity is reflected by their high dielectric constants. These solvents will therefore extract chloroquine and its metabolites whereas ether is capable of extracting the desethyl chloroquine, and heptane tends to extract chloroquine exclusively; these facts were confirmed on a TLC plate. The percentage recoveries of chloroquine from plasma when chloroform was used as the extracting solvent were almost nearing those of ether but not quite as high. Of the 5 organic solvents considered in this investigation, diethylether proved to be the best solvent for extraction of chloroquine.

TABLE 1

Percentage recoveries of chloroquine from plasma using different organic solvents

Amount added (μg/ml)	Diethyl-ether	Heptane	Dichloromethane	Dichloroethane	Chloroform
1.0	95.8 ± 2.8	85.7 ± 4.8	75.7 ± 5.7	73.6 ± 4.9	82.7 ± 4.6
0.5	96.0 ± 2.2	87.1 ± 5.0	76.8 ± 6.4	75.0 ± 5.1	83.5 ± 5.2
0.1	95.4 ± 2.5	84.2 ± 4.1	74.9 ± 5.2	72.3 ± 6.4	80.5 ± 3.8
0.05	95.9 ± 3.0	86.9 ± 5.1	77.0 ± 4.5	75.0 ± 7.3	81.3 ± 4.8
0.01	94.6 ± 3.5	75.9 ± 4.5	–	–	–
0.005	95.2 ± 3.8	–	–	–	–

Each value is the mean of 10 determinations ± standard deviation.

The high percentage recoveries obtained in the case of diethylether show that the drug partitions better into the ether phase than the other solvents. It is expected that heptane should also be a solvent of choice but because of the high blank reading it will be difficult to recommend this solvent. This problem which is associated with heptane has further confirmed the work reported by Offerhaus and van der Vecht (1976) who had shown that acid extracts from heptane caused high blank readings in fluorimetric analysis of drugs. This might be due to light scattering droplets or deposition of solvent films on the inside of the cuvettes during fluorimetric analysis.

Recent developments in the use of chloroquine as an antimalarial agent have placed much importance on the pharmacokinetics of the drug (Adelusi, 1982) and diethylether has been found to be the most useful organic solvent for the extraction of chloroquine from body fluid. This investigation therefore suggests the use of ether as an extraction solvent particularly when the sensitivity of the method is important.

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