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Research Article

Quantification of Chloroquine (Free Drug) in Liver Tissues by GC-MS

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Abstract

Chloroquine (CQ) which belongs to the 4-aminoquinoline group of compounds and one of the most antimalarial. Chloroquine has been studied for the treatment and prevention of coronavirus disease 2019 (COVID-19), during treating coronavirus disease 2019 (COVID-19) some big side effects were noted. Quantification method involves a protein precipitation and liquid - liquid extraction procedure, analyses from alkaline and acidic extractions before GC/MS analysis and comparisons by two thermal program gas chromatography, first method thermal analyzing prefer than second method and alkaline is more quantified than acidic extraction.

Keywords: Chloroquine; Metabolites; Toxicity; GCMS

Objective

The work contains suitable method of extraction and comparison between two thermal programs were used in GCMS.

Introduction

Chloroquine very slightly soluble in water, soluble in chloroform and ether and has structure N -(7-Chloro–4–quinolinyl)-N^1,N^1-diethyl–1,4– pentanediamine (Figure 1).

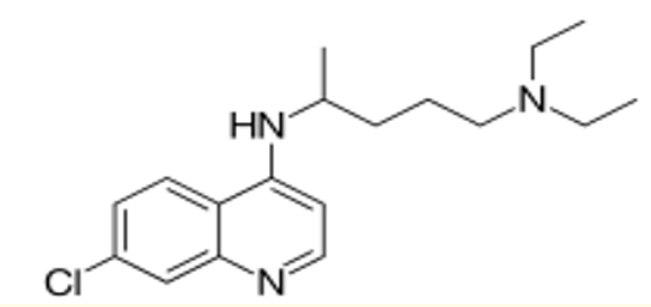

Figure 1: Chemical structure of Chloroquine.

Chloroquine (CQ) used for decades to prevent or treat malaria caused by mosquito bites. Chloroquine belongs to a class of drugs known as antimalarials. Chloroquine is known since 1934, intoxications due to chloroquine overdose are rare in countries.

Chloroquine disposition in the body is rapidly absorbed after oral administration and widely distributed into body tissues. It accumulates in high concentrations in the kidneys, liver, lungs, spleen and is strongly bound in melanin–containing cells (eyes and skin). It also crosses the placenta. Metabolic reactions include N-dealkylation and deamination followed by conjugation, possibly with glucuronic acid, of the carboxylic acid metabolites; the metabolites include monodesethyl- and didesethylchloroquine, 4-(7–chloroquinol–4–ylamino)pentan–1–ol, and 4- (7–chloroquinol–4–ylamino)pentanoic acid and its conjugate, Plasma half– life about 25 to 60 days [1-5].

Toxicity Symptoms in overdose manifest rapidly (minutes to hours) and cardiotoxicity such as cardiovascular shock and collapse are most prominent. Neurotoxic effects such as psychosis and seizure may also occur [6].

In 1888 Franz Hofmeister primary determined the capability of different salts to stabilize proteins from water, which depends on the types and concentration of the salts [7]. This effect is classified as the Hofmeister series (HS), lyotropic sequences, or ion specificity series and is a very widespread phenomenon [8].

According to the ability of salts to influence the solubility of proteins in aqueous solution, ions can be divided into "salting-in" or "salting-out" ions [9].

Chemicals and reagents

- Samples of chloroquine were kindly donated. Ammonium sulfate and hydrochloric acid, aqueous ammonia, chloroform purchased from Algomhoria Co. All other chemicals were of analytical quality and commercially available.
- Standards of the stock standard solution of 100 µg/ml chloroquine was prepared in a calibrated flask

Apparatus

For the quantitative determinations, a GC MS was used.

Sample preparations

Liver specimens were collected at autopsy. Liver homogenates were prepared by adding saturated ammonium sulfate acidified by hydrochloric acid to overnight and filtrate then acidic extraction after that converting filtration to alkaline medium by ammonia, extraction by chloroform.

Instrumental

Gas chromatography with column HP 5-MS, (0.2 um × 30m × 0.25 µm) non-polar chromatographic capillary column (Agilent Technologies). (Agilent column 190915-433UI) coupled to a mass spectrometry detector (Agilent 5977). Constant flow Gas type: Helium. The separation and identification was performed by applying the following thermal programs: Table 1 thermal programs.

Thermal program method (first)	Thermal program method (second)
Run time 24 min Resolution mode: Scan Oven condition: Setpoint 55°C Hold time 0.7 min Post run 300°C Ramp 20°C/min Value 95°C Ramp 10°C/ min Value 300°C Hold time 7.75 min Max temp. 325°C ALS condition: Injection volume 1 uL Setpoin (initial) 280 °C Mode splitless Pressure 13.03 Psi Helium flow: 1.5 ml/min MSD transfer: Setpoint (initial) 300 °C Retention time: 14.87 min. ± 2%	Run time 21.5 min Resolution mode: Scan Oven condition: Setpoint 50°C Hold time 1 min Post run 300°C Ramp 20°C/min Value 200°C Ramp 10°C/min Value 300°C Hold time 3 min Max temp. 325°C ALS condition: Injection volume 1 uL Setpoin (initial) 250°C Mode splitless Pressure 7.65 Psi Helium flow: 1 ml/min MSD transfer: Setpoint (initial) 250°C Retention time: 17.86 min. ± 2%

Table 1

Calibration work

MassHunter creates a calibration curve by plotting response ratio vs. concentration. To determine the concentration of a sample, the response ratio is determined and the concentration can be calculated using the regression equation for the curve. Six concentrations (0.01, 0.5, 10, 20, 50 and 100 ug/mL) of the analytes were prepared and analyzed in three different analytical runs. The presence of quantifier ions m/z values and qualifier ions m/z values at their respective retention times was required to deem a calibration point usable for the determination of the calibration model and subsequent studies.

Limit of detection (LOD)

The LOD is an estimate of the lowest concentration of analyte in a sample that is reliably differentiated from the signal due to the blank matrix and identified by the analytical method used, LOD may be estimated from a minimum of three linear calibration curves constructed over the working concentration range over different runs.

Precision: Precision is the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogenous sample. The same data from the bias study was used to evaluate within-run and between-run precision.

Result and Discussions

The extraction of drugs from solid tissues requires the tissue matrix be broken down to release drugs into an environment

from which they are accessible for solvent extraction. This can be achieved by cutting tissues by scissor and homogenization. Many methods direct solvent extraction are used in blood and urine but in tissues direct methods of extraction not suitable, So protein precipitation Liquid-liquid extractions are predominates in most laboratories, The choice of an appropriate solvent is often a matter of experience or tradition. The chosen solvent should ideally extract as much of the target analyte as possible.

Many methods of extraction drugs from biological samples were used, but the most popular ones is Salt Induced Precipitation (Salting Out). with ammonium sulfate "salting out" precipitation protein can occur with a number of different types of neutral salts but ammonium is preferred salt because it is high on the Hofmiester series and has a high solubility rate.

Protein interacts with the salt as opposed to the water, which leads to less interaction between the water and the protein's solvent layer, which in turn leads to more hydrophobic patches being exposed and to encourage those patches to interact with one another. This leads the proteins to aggregate and precipitate.

The screening of the postmortem liver specimen for pesticides and insecticides, other toxic compounds, opiates, benzolyecgonine, benzodiazepine derivatives, amphetamines, cannabinoids, propoxyphene, tramadol and other routine analyses was negative results Figure (2, 3). The results in an efficient chromatography for chloroquine alkaline extraction the retention time is 14.78 min and chloroquine acidic extraction 17.38 min. The results in an efficient chromatography retention time for chloroquine standard is 14.84 min according to the first program thermal, the main Principal ions fragmentation of chloroquine is m/z 58, 73, 86, 87, 112, 245, 247, 319.

Figure 2: Alkaline extraction.

Figure 3: Acidic extraction.

The work is determining the chloroquine in postmortem specimens and comparison between two methods of thermal program. The analytes were extracted from alkalinized samples after protein precipitation before GC/MS analysis.

Concentration of tissue liver was determined 850 u/g in alkaline extraction by first thermal method and retention time at 14.78 min, while concentration of tissue liver was determined 520 u/g in acidic extraction by second thermal method at retention time 17.38 min.

The fragmentation ions in alkaline medium complete and more appearances than acidic extraction, so that the first thermal program suitable and favorable in qualitative and quantification.

The proposed method resulted in a procedure most useful in cases of deliberate poisoning with the anti-inflammatory and antimalarial drug chloroquine.

Conclusion

Chloroquine drug can be risky to some extent if misused. Their continuous use or an overdose can cause serious complications that could prove fatal, so dose of these drugs must be under revision of physician continuously. Method of protein precipitation by ammonium sulfate acidified by hydrochloric acid and extraction in alkaline medium by chloroform is favorable and applied first thermal program method is suitable.

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