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Simple and Direct Quantitative Analysis for Quinidine Drug in Fish Tissues

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Abstract : Analysis of quinidine for fish tissues using single drop microextraction (SDME) coupled with atmospheric pressure matrix assisted laser desorption/ionization mass spectrometry (AP-MALDI-MS) are reported. Optimization conditions; such as extraction solvent, extraction time, pH of the aqueous solution, salt additions (NaCl), stirring rate, matrix type and concentration are investigated. Linear dynamic range (μM), limit of detection, relative recovery%, and enrichment factor are 0.08-9.2, 0.05, 94.8±3.1-98.5±3.3%, 4.34±0.28-4.40±0.30, respectively. SDME-AP-MALDI-MS shows good intraday and interday reproducibility.

Keywords : quinidine; fish tissues; environmental analysis; quantitative analysis; extraction; SDME-AP-MALDI-MS.

Introduction

Drug analysis is paramount important for environmental concerns and human being. Analysis of the drug levels is vital for medicine, clinical and forensic toxicology, as well as for the monitoring of therapeutic drug. Annual Report of the American Association of Poison Control Centers in 2012 reported that more than 3.37 million (2,275,141 human exposures, 66,440 animal exposures, 1,025,547 information calls, 5,679 human confirmed nonexposures, and 218 animal confirmed nonexposures) regarding unintentional and intentional exposures were recorded.[1] There was a decline in the exposure percentage (7.7% less than that was reported in 2009). But, human exposures with more serious outcomes were increased to 4.5%.[2] Thus, simple, effective and cheap methods are highly demanded.[3] Among many analytical techniques, mass spectrometry is sensitivity, simple, and offers direct analysis.[3-6]

Quinidine (QD), (S)-(6-Methoxyquinolin-4-yl)((2R,4S,8R)-8-vinylquinuclidin-2-yl)methanol, is a pharmaceutical agent that acts as a class I antiarrhythmic agent (Ia) in the heart.[7] It causes many side effects, block many enzyme and inhibit the transport of protein such as P-glycoprotein,[7] interact with drugs[8] and serum albumin.[9] These interactions are useful for separation.[10] Quinidine has been determined by tritium nuclear magnetic resonance (NMR),[11] capillary electrophoresis,[12] DNA-based nanocom-posite as electrochemical chiral sensing platform,[13] ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometer system,[14] and others.[15,16] However, these techniques are expensive, required sophisticated sample preparation, lack of sensitivity, and cannot be used for quantitative analysis.

Mass spectrometry based techniques such as matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) is soft analytical tool.[17] MALDI-MS provides soft ionization for several analytes such as metals,[18,19] metallodrugs,[20] proteins,[21-23] pathogenic bacteria[21,24-36] and others.[37-39] MALDI-MS is sensitive, simple, high throughput and soft analytical method.

Herein, a simple, direct and quantitative analysis of quinidine was reported. The analysis take placed using single drop microextraction (SDME) coupled with atmospheric pressured MALDI-MS (AP-MALDI-MS). Extraction parameters, such as solvents, extraction time,

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pH values, salt addition, centrifugation time and matrix concentration, were optimized. Under optimized conditions, the approach has been applied for quantitative analysis of quinidine in real sample e.g. fish tissues using internal standard approach (ISA).

Experimental

Chemicals and materials

HPLC grade of solvents including toluene, octanol, xylene, n-hexane, iso-octane and methanol were purchased from Riedel-de Haen Company (Seelze, Germany). Quinidine, cinchonidine, α-cyano-4-hydroxycinnamic acid (CHCA), and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (NaCl) was purchased from Merck (Darmstadt, Germany). Fish (carassius auratus) was obtained from local pet store in Kaohsiung, Taiwan.

Preparation of fish extracts

Quinidine (3-8 mg/kg) was added to the fishes (2.2-4.3 g) tank for 10-15 hours. Then, the fishes were transferred into deionized water for 30 min. The fish was dried and grounded homogenously using mortar and pestle. About 2.0 g of fish was diluted with 1 mL of methanol. After ultrasonication for 10 min, the solid portions of fish extracts were removed.

Single Drop Microextraction

SDME extraction was performed following these steps: 1 mL of the liquid portion of fish extracts was diluted with 9 mL of deionized water in a glass vial (25 mL) and placed on a magnetic plane. The aqueous solution was agitated using a magnetic stirrer with a constant stirring rate. Using the plunger of microsyringe (10 μL), 1 μL of the organic solvent was depressed into the aqueous phase. After extraction within certain time, the organic solvent was

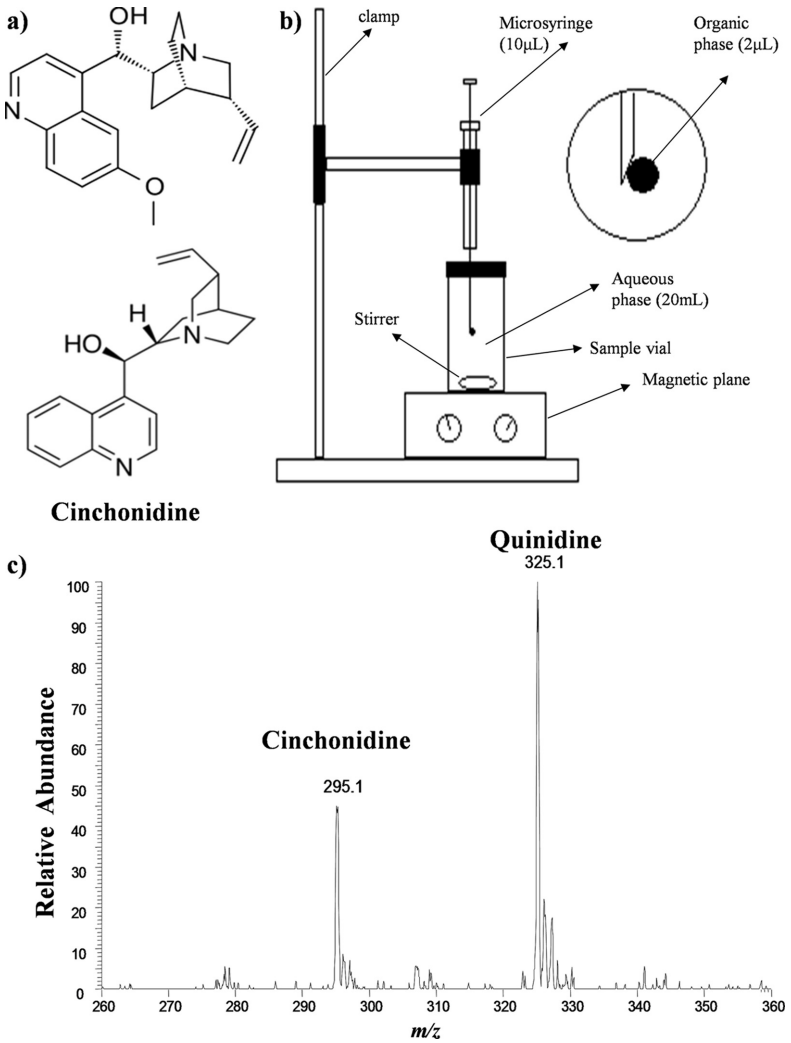

Figure 1. a) Chemical structures of quinidine (QD) and cinchonidine (CD), b) SDME set up, and c) AP-MALDI-MS pattern of quinidine (QD, 325): cinchonidine (CD, 295).

retraced carefully. Internal standard solution of cinchonidine was added to the organic drops and then analyzed using AP-MALDI-MS.

Instruments

All experiments were performed in ThermoFinnigan LCQ (ion trap mass spectrometer) equipped with an AP-MALDI source. Samples were ionized using nitrogen laser (wavelength 337 nm, positive mode) under atmospheric pressure with the following parameters; injection time, capillary temperature, capillary voltage, tube lens offset, attenuation of laser power and laser shots are 1070 ms, 250°C, 40 V, 70 V, 60%, and 200 respectively.

Results and Discussion

Extraction and analysis of quinidine (QD) in water and fish tissue are carried out using SDME coupled AP-MALDI-MS. The quantitative analysis of quinidine take placed using internal standard method (cinchonidine, CD) (Fig. 1a). For the quantitative analysis, the ratios of the signal of the two compounds ($I_{QD,325}$, $[QD+H]^+$ = 325): $I_{CD,295}$ (CD, $[CD+H]^+$ 295) are used. SDME is simple, fast, and inexpensive technique. SDME requires simple vial and microsyringe as shown in Fig. 1b. AP-MALDI-MS is selected as detection method. AP-MALDI-MS has high sensitivity offers high-throughput analysis, and requires tiny amount of the sample. The latter advantage is in rhythm with AP-MALDI-MS that required very tiny volume (ca. 10 μL). In order to reach high sensitivity; parameters conditions of extraction and analysis using AP-MALDI-MS are highly required. Parameters, such as extraction solvents, extraction time, pH effect of the aqueous solution, salt (NaCl) additions, centrifugation time and matrix concentration (CHCA), are optimized. The selection of these parameters is evaluated using the ratio intensity quinidine: cinchonidine. The summary of these data are tabulated in Table 1.

Optimization of Extraction Conditions

Optimization of Extraction Solvent

The mass transfer of quinidine is based on the affinity to organic solvent. Several immiscible organic solvents including toluene, xylene, n-hexane, iso-octane and octanol were investigated (Table 1). Data show that toluene is the best extraction solvent (Table 1, Fig. 2a). Therefore, toluene is selected for further quantitative experiments (Fig. 2a).

Extraction Time

Extraction time (1, 3, 5, 7, 9, and 11 min) were tested as shown in Fig. 2b. The ratio intensity (I_{325}/I_{295}) increase with the increase of extraction time and reach maximum at 5 min. The intensity after 5 min decrease due to the lost of the analyte. Thus, 5 min is selected as optimized extraction time. Data (Fig. 2b) indicate that SDME is fast microextraction technique for quinidine from aqueous solution.

Table 1. Optimization parameters for the SDME-AP-MALDI

Optimized condition	Parameters	I_{325}/I_{295} (n=3)
Selection of solvents	Toluene	0.83±0.04
	Xylene	0.48±0.02
	n-hexane	0.33±0.01
	iso-octane	0.58±0.04
Extraction time (min)	1	0.42±0.02
	3	0.45±0.02
	5	0.48±0.02
	7	0.53±0.03
	9	0.49±0.03
	11	0.48±0.03
pH of aqueous phase	4	0.12±0.01
	6	0.28±0.01
	8	0.51±0.02
	10	26.12±1.31
	12	23.71±1.23
Salt addition (NaCl)	0%	14.73±0.81
	5%	9.02±0.44
	10%	4.60±0.23
	20%	1.72±0.09
Stirring rate (rpm)	0	0.12±0.01
	50	0.18±0.01
	100	0.51±0.02
	150	0.23±0.01
	200	0.28±0.02
Matrix C (ppm)	5000	2.6±0.18
	10000	3.1±0.19
	15000	5.3±0.25
	20000	7.6±0.31

pH solution of the aqueous solution

The pH of aqueous solution affect the microextraction. pH values in the range of 4-11 was investigated (Fig. 2c, Table 1). Acidic pH cause protonation of the compound. The protonation increases the affinity of the compound to aqueous solution. This feature lead to low microextraction efficiency (Fig. 2c). In contrast, basic pH show higher microextraction efficiency (Fig. 2c). Data show that pH value of 10 is the optimized pH value of the aqueous solution.

Salt Additions (NaCl)

Addition of salt such as NaCl affect the extraction and detection of quinidine. The ionic strength of aqueous solution affect the microextraction procedure of quinidine. The salt addition changes the ionization of the target analyte using AP-MALDI-MS. Presence of salt may cause ion suppression. Different percentage of NaCl (0, 5, 10, 20%) were tested as shown in Fig. 2d. Data shows that the

intensity ratio of I_{325}/I_{295} decrease with the increase of the salt content. The decrease of the intensity may be due to the decrease of the extraction efficiency or due to ionization suppression. It is important to mention that adduct of the target analyte with Na^+ ions was not observed (Fig. 1c, the peak of $[QD+Na]^+$ = 347 is absent).

Stirring Rate

Stirring rate during SDME affect the extraction efficiency. Stirring increase the mass transfer of the target species from aqueous solution to the organic solvent (toluene). Static and dynamic effect has been investigated as tabulated in Table 1. Data show that the ratio of intensity (I_{325}/I_{295}) increase with the increase of stirring rate and reach maximum at stirring rate 100 rmp. The stirring at high rate shows decrease of extraction due to lost of the toluene droplet.

Detection Method

Matrix Type, Additives and Concentration

The optimization of detection method (AP-MALDI-MS) was investigated. CHCA is selected as the optimal organic matrix.[40,41] CHCA is suitable matrix for small molecular weight (< 2000 Da). Data (not shown here) shows that 20000 ppm is the optimium matrix concentration.

Calibration Curve for Water Solution and Fish Tissues

Under the optimal conditions, extraction solvent is

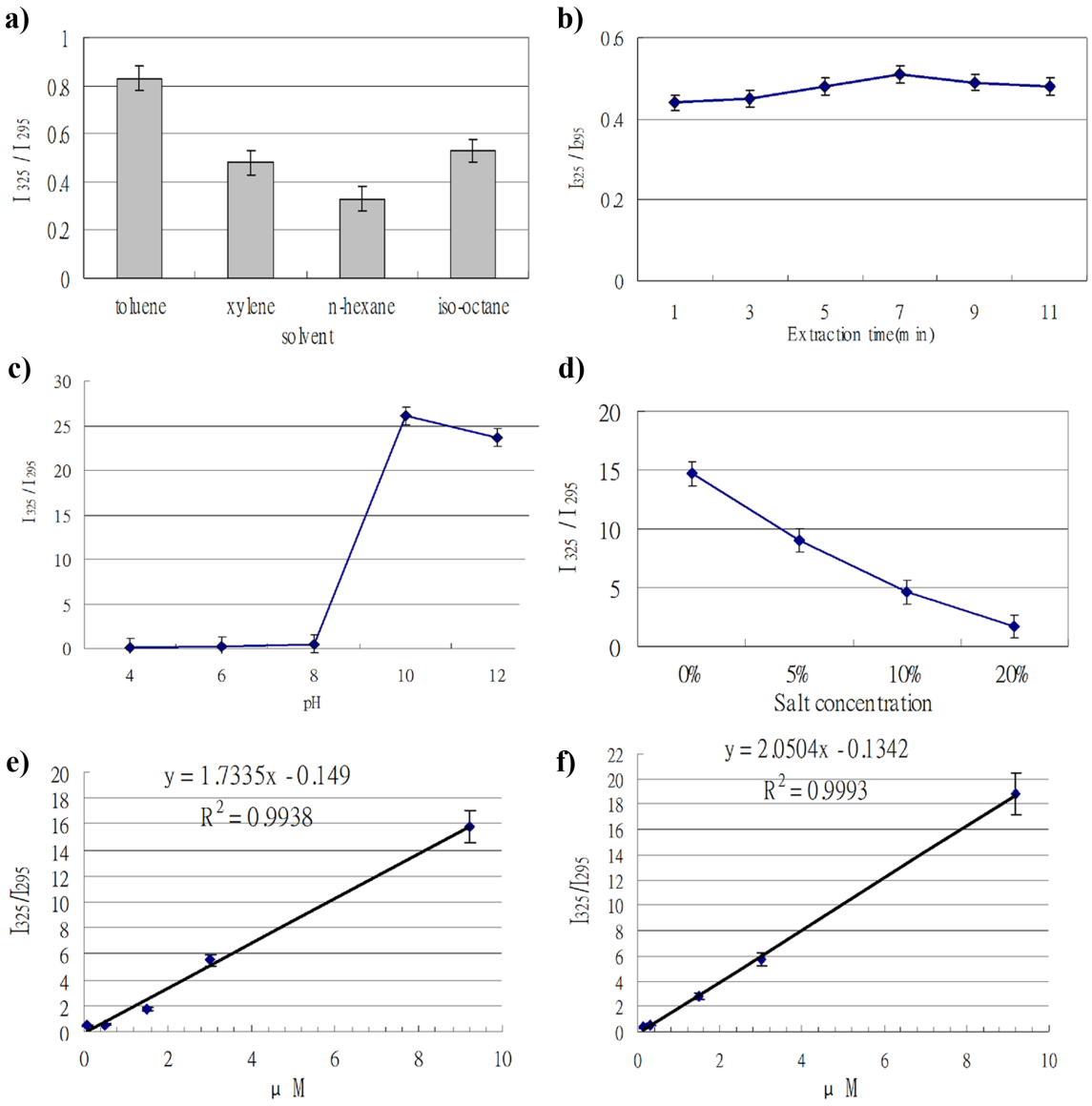

Figure 2. Optimization of a) extraction solvent, b) extraction time, c) pH of the aqueous solution, d) salt concentration, and Calibration curves for the SDME coupled to AP-MALDI-MS method applied in (e) deionized water and (f) fish samples under the best optimization parameters.

Table 2. Linearity, correlation coefficient and LODs.

Sample	Linear Range (μM)	R^2	LOD (μM)
Water	0.08-9.2	0.9938	0.05
Fish	0.12-9.2	0.9993	0.08

Table 3. Interday/intraday for SDME- AP-MALDI-MS.

Sample	(μM)	Intraday RSD (%)	Interday RSD
Water	1.5	6.7	8.8
	3.0	8.6	8.9
	9.2	8.6	8.9
Fish	1.5	9.4	9.4
	3.0	9.2	9.5
	9.2	9.1	9.6

Table 4. Relative recovery and enrichment factor (EF).

Sample	C (μM)	Recovery (%)	EF (n=5)
Standard Solution	1.5		4.54±0.31
	3.0		4.43±0.29
	9.2		4.59±0.32
Fish Tissue	1.5	94.8±3.1	4.38±0.28
	3.0	91.8±2.8	4.34±0.28
	9.2	98.5±3.3	4.40±0.30

Table 5. Monitor the adsorption of the drug in fish.

Solution (mg/kg)	I	Fish (mg/kg)	Adsorption%
3	0.97	2.16	72
5	1.68	3.75	75
8	2.72	6.18	77

SDME-AP-MALDI-MS is successfully applied for the analysis of quinidine in fish tissues. Data shows good calibration curve for the analysis of water and fish tissue. SDME-AP-MALDI-MS is simple, sensitive, applicable for real sample, reproducible, shows high recovery and enrichment factor and can be extended to other real samples.

toluene, extraction time is 7 min, stirring rate 100 rmp, CHCA as matrix with concentration 20000 ppm and minimum salt addition, calibration curve for analysis of quinidine in water (Fig. 2e) and fish tissue as real sample are (Fig. 2f). Figure 2 shows a linear relationship for water and fish sample with regression coefficient 0.99 and 0.99, respectively. The linear dynamic rage, limit of detection and R^2 were tabulated in Table 2. Data indicate high efficiency of the extraction procedure for simple solution such as water and complicated sample such as fish tissues. The interday and intra reproducibility are critical for SDME and AP-MALDI-MS analysis (Table 3). Data show high reproducibility for interday and intraday. Data show that the current approach offer high recovery with brilliant enrichment factor (Table 4). The data indicates high reproducibility of the current approach and wide applicability of this method for quinidine drug for fish tissues.

Application of the SDME-AP-MALDI-MS for the Adsorption of Quinidine in Fish

The adsorption of the drug in fish was monitored using the described method and tabulated in Table 5. Different concentration of the drug (3, 5, and 8 mg/Kg) were evaluated. The concentration of quinidine in fish are 2.16, 3.75 and 6.18 that corresponding to 72, 75 and 77%, respectively.

Comparison with other techniques

The current method is direct, simple, showed good interday and intraday reproducibility and offers a quantitative analysis for quinidine. In general, mass spectrometry introduce direct analysis compared to many other techniques.[42] Vibrational spectra of quinine, quinidine, cinchonine, and cinchonidine were reported.[16] This approach required theoretical calculations and special technique such as Raman Optical Activity (ROA). Using normal Raman spectra, all these alkaloids in solution exhibited similar patterns and cannot be used for differentiation.[16] In contrast, MS spectra differentiate between these species based on its molecular weight. Furthermore, ROA cannot be used for quantitative analysis. Quinidine has been determined by tritium NMR.[11] The technique is sophisticated and expernsive. Capillary electrophoresis[43,44] is very sensitive to the matrix effect and require laborius optimization. DNA-based nanocomposite as electrochemical chiral sensing platform,[13] and ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometer system[14] were used for quinidine analysis. These techniques are expensive and require trained person.

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