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Sequential Injection Lab-at-Valve Micro-Solvent Extraction Assay of Tetracycline

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Abstract

Sequential injection lab-at-valve (SI-LAV) micro-solvent extraction determination procedure for tetracycline hydrochloride in pharmaceutical analysis is proposed. It is based on the formation of a yellow colored ion associate of a protonated cation of tetracycline ($TCLN^+$) with anionic form of bromocresol green (BCG), in phthalate buffer pH 4, which could be extracted into dichloromethane. The organic phase is separated from the aqueous phase by means of a conical separating chamber in lab-at-valve unit which placed in one port of the selection valve. An extracted organic layer containing $TCLN^+$-BCG^- associate is monitored by a fiber optic spectrophotometer. The procedure leads to micro-volume analysis. This is a green analytical chemistry. The proposed method was applied to the assay of tetracycline hydrochloride in pharmaceutical samples.

Keywords Sequential injection, lab-at-valve, micro-solvent extraction, ion associate, tetracycline

1. Introduction

Tetracycline hydrochloride is a well-known antibiotic molecule belonging as a group of tetracyclines that have been used for drug for treatment of infections caused by microorganisms.

Extensive literatures are available and numerous methodologies have been developed for analytical purposes, including spectrometry [1], voltammetry [2], fluorometry [3-5], FT-MIR, FT-NIR [6] and HPLC [7-16].

Some of the methods require highly skilled personnel for operation and/or make use of instruments that are often expensive for routine laboratories, especially in the developing countries.

Prior to analysis, the antibiotics in various kinds of samples, including biological fluids, may require separation. Liquid-liquid extraction is one of the most effective means for transferring of tetracycline from aqueous phase to organic phase. Extraction of tetracycline based on ion-association reaction using organic dyes as the counter ions has been reported [17]. However, conventional extraction procedures have suffered from the disadvantages of high consumption of sample and toxic organic solvent, low sampling frequency, loss of analyte through manipulation and contamination of atmosphere by organic vapor.

Various automated and miniaturized liquid-liquid techniques, such as flow injection (FI) [18-20], sequential injection (SI) [21] and microfluidic [22] have been developed to overcome the drawbacks. For handling organic solvents, SI-systems have shown significant advantages over traditional FI systems, with simplicity of manifold design, robustness and versatility.

SIA with a simple approach called lab-at-valve (LAV), firstly introduced by our group [23-28], is another approach for SIA which becomes an alternative cost effective micro total analysis system.

A simple, robust manifold design brings sequentially loaded organic solvent and aqueous sample into contact, and later resolves them without the conventional flow injection extraction components of phase segmentator and separator. The phenomena happening there, due to gravity, are similar to that of batch conventional operation in a separatory funnel but down-scaling and automation. Moreover, the SI-LAV unit can be designed to be easily attached to some ports of the selection valve. It can take a function like a lab-on-valve (LOV) unit. SIA-LAV is then different from SIA-LOV in that a simple-to-made-device is plugged into one of the ports of a selection valve without any taking apart of any component of the selection valve as it does for LOV [23-28]. This approach was successfully demonstrated for determination of chloride [24], assay of hyaluronan (HA) in serum sample [25], diphenhydramine hydrochloride [26] and surfactant [26-27].

In the present work, the SIA-LAV approach is proposed as a novel alternative for simple automated on-line liquid–liquid micro-extraction based on ion-association reaction of a protonated cation of tetracycline with anionic form of organic dye, bromocresol green (BCG). The procedure leads to micro-volume analysis. This method was applied to the assay of tetracycline hydrochloride in pharmaceutical samples.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical grade. Deionized water was used for the preparation of all solutions.

Tetracycline hydrochloride (1000 ppm) was prepared by 0.1 g of tetracycline hydrochloride by DI water and making volume to 100 mL.

Phthalate buffer (0.1 M, pH 4) was prepared by mixing of 100 mL of 0.1 M potassium hydrogenphthalate and 40.6 mL of 0.1 HCl. The solution was adjusted to pH 4 by HCl or NaOH.

Bromocresol green solution ($1x10^{-3}$ M) was prepared by

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dissolving 0.0698 g of bromocresol green powder (acid form) in 2 mL of 0.1 M NaOH and diluted to 100 mL with a phthalate buffer of desired pH.

2.2. SI-LAV set-up

The schematic diagram of the SI-LAV for the micro solvent extraction system is presented in Fig. 1. The system consisted of a 2.5 mL syringe pump (USA), a 10 port selection valve VICI with a microelectric actuator (Valco Instruments, USA), a light source model LS-1LL tungsten halogen lamp and a USB2000 spectrometer (Ocean Optics Inc., USA). The syringe pump was connected to the center of the selection valve by means of an extraction coil (PTFE tubing, Upchurch, USA). A separating chamber was modified from a 1 mL pipette tip situated in a fiber-optic spectrophotometer. The FIAlab® software (FIA instruments, USA) was used for the instrument control and data acquisition. The data processing was computed by using Microcal Origin 7.0. For batch experiment, spectrophotometric measurements were made with UV-Vis spectrophotometer model Cecil CE1010 (Cecil Instruments Ltd, England). pH measurements were done with pH meter model 744 (Metrohm, Switzerland).

2.3. SI-LAV procedure

2.3.1. SI-LAV for single extraction procedure

The SIA lab-at-valve micro-solvent extraction system operates in three major operation stages. First, sample, reagent and organic solvent were sequentially aspirated into a coil which is attached to a central port of a conventional multi-position selection valve. Second, the extraction step was performed by using one cycle of flow reversal. The aqueous and organic phases were then separated in a conical separating chamber. The final step is cleaning of the separating chamber and extraction coil before the next analysis. The operational sequence of the SI-LAV is listed in Table 1.

2.3.2. SI-LAV for double extraction procedure

The same volume ratio of sample to reagent was applied for both. Double extraction was performed by having an extraction coil (auxiliary coil) on port 2 of the selection valve (Fig. 2). Portions of 200 µL of TC, 200 µL of bromocresol green and 200 µL of dichloromethane were aspirated into the extraction coil. After using of one cycle of flow reversal, the solutions, the aqueous and organic were then dispensed to the auxiliary coil. Then, TC, bromocresol green and dichloromethane in the same proportions were done in the same as the first operation stage. The solutions were then dispensed to the separating chamber. After that, the solutions which were in the auxiliary coil were then aspirated into EC and were dispensed into the chamber. Absorbance was then monitored spectrometrically.

2.4. Procedure for tablets

Twenty tablets of tetracycline hydrochloride were accurately weighed and the average weight of tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalent to 250 mg tetracycline hydrochloride was dissolved in the deionized water. The resulting solutions were shaked in an ultrasonic bath for 5 minutes. Clear solutions were analyzed by SI-LAV micro-solvent extraction.

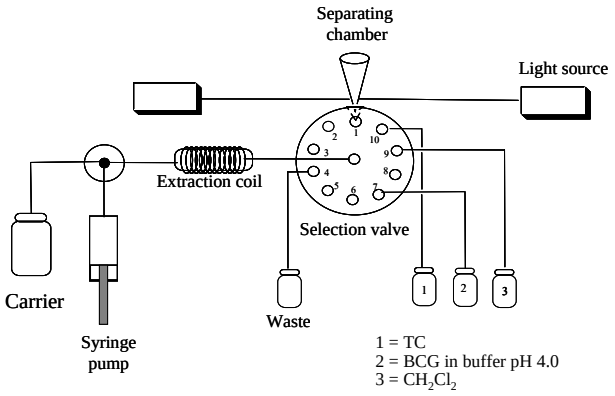

Fig. 1 Schematic diagram of SIA system.

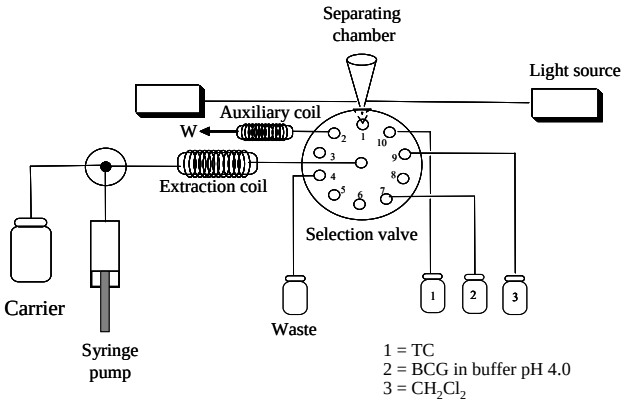

Fig. 2 SI-LAV micro-extraction for double extraction system.

Table 1 Operational sequence of SI-LAV system for the determination of tetracycline hydrochloride

Sequence	Valve position	Mode	Volume (µL)	Description
1	10	Aspirate	400	Aspirate 200 µL TC into EC
2	7	Aspirate	200	Aspirate 200 µL BCG into EC
3	9	Aspirate	200	Aspirate 200 µL CH_2Cl_2 into EC
4	10	Aspirate	200	Aspirate 200 µL TC into EC
5	7	Aspirate	200	Aspirate 200 µL BCG into EC
6	9	Aspirate	200	Aspirate 200 µL CH_2Cl_2 into EC
7	1	Dispense	2400	Extract
8	1	Aspirate	1000	Extract
9	1	Dispense	2000	Extract
10	1	Delay 5 s	-	-
11	1	Aspirate	150	-
12	1	Delay 5 s	-	Absorbance detection
13	1	Dispense	1500	Clean LAV unit
14	1	Aspirate	1500	Clean LAV unit
15	4	Dispense	Empty	Waste
16	1	Aspirate	1800	Clean LAV unit
17	1	Dispense	1800	Clean LAV unit
18	4	Dispense	Empty	Waste
19	Valve in	Aspirate	2500	Fill syringe with DI water
20	4	Dispense	Empty	Clean EC

3. Results and discussion

The principle of this is based on that ion-pairs are formed between tertiary amino group tetracycline hydrochloride drugs and bromocresol green reagent *via* the protonated nitrogen atom of the drug (Fig. 3). Tetracycline hydrochloride can be protonated under an acidic condition. On adding basic drug solution, a stable yellow ion-pair is formed in acidic medium of pH 4.0. The ion-pair formed is extracted in dichloromethane. Different factors affecting these reactions were studied and established.

$$TCLN^{+}_{(aq)} + BCG^{-}_{(aq)} \rightleftharpoons TCLN^{+} - BCG^{-}_{(aq)}$$
$$TCLN^{+} - BCG^{-}_{(aq)} \xrightleftharpoons{Extr.} TCLN^{+} - BCG^{-}_{(org)}$$

Fig. 3 Reaction schemes of TC and BCG.

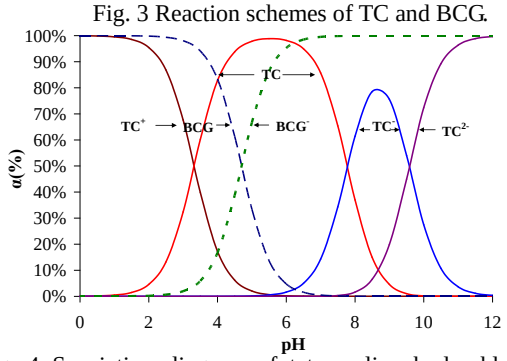

Fig. 4 Speciation diagram of tetracycline hydrochloride as a function of pH [29] (α = fraction).

3.1. Absorption spectra

In the preliminary studies, batch wise experiments were performed; yellow color of ion-pair compound in the organic layer was recorded from 400 to 700 nm by using the fiber optic USB 2000 spectrophotometer. The various measurement wavelengths were investigated by comparing of the calibration curves. The wavelength at 425 nm was used for subsequent work due to obtaining a good calibration with appropriate slope and correlation coefficient.

3.2. Effect of pH

The protonated tetracycline hydrochloride is associated with a negatively charged bromocresol green and forms the ion-pair compound which can be quickly and efficiently extracted into a slightly polar solvent such as dichloromethane. Tetracycline shows three association constants of approximately 3, 7 and 9 [29] as the dissociation forms shown in Fig. 4, whereas the pK_a value of bromocresol green is 4.7 [30]. The desired pH condition should provide highly protonated form of tetracycline hydrochloride and negatively charged bromocresol green to form ion-association. In this work, pH 4.0 was selected.

3.3. SI-LAV optimization

3.3.1. Sequence order

In the proposed SIA system (Fig.1), the main variables affecting the extraction and detection of ion-pair were investigated. Sequence orders of aspiration are optimized. The two different sequence orders were studied with large and small segments of the solutions (Fig. 5). Good mixing was obtained by using of small segments of the solutions presented by lower %RSD than that obtained by the sequence using the large segments volume.

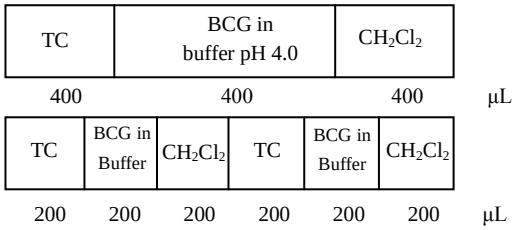

Fig.5 Sequence orders for the determination of tetracycline hydrochloride in SIA system.

3.3.2. Number of flow reversals

The number of flow reversals was varied from 1 to 4. It was observed that there were not significantly different signals when increasing of number of flow reversal. One cycle of flow reversal was selected for sufficient sampling efficiency.

In order to increase sensitivity and precision of analysis, three methods of analysis were examined, namely (1) Mixing of TC and bromocresol green before extraction with dichloromethane (2) Single extraction and double extraction (3) Increasing of sample volume.

3.3.3. Mixing of TC and bromocresol green before extraction with dichloromethane

The sequence order sample, bromocresol green and dichloromethane was used for aspiration and subsequent extraction in the SIA lab-at-valve system. Using this sequence order was compared with mixing of tetracycline hydrochloride and bromocresol green with one cycle of flow reversal before subsequent aspiration of dichloromethane for extraction. It can be seen that there were not significant differences between the two methods.

3.3.4. Single extraction and double extraction

Two extraction methods were investigated, one involving single extraction, and another is double extraction using the SI-LAV system as presented in Fig. 2. The same volume ratio of sample to reagent was applied for both. Double extraction was performed by having an extraction coil (auxiliary coil) on one port of the selection valve. Portions of 200 µL of TC, 200 µL of bromocresol green and 200 µL of dichloromethane were aspirated into the extraction coil. After using of one cycle of flow reversal, the solutions, the aqueous and organic, were then dispensed to the auxiliary coil. Then, TC, bromocresol green and dichloromethane in the same proportion was done in the same of the first operation stage. The solutions were then dispensed to the separating chamber. After that, the solutions which were in the auxiliary coil were then aspirated into EC and were dispensed to include with the solutions in chamber. Absorption was then monitored spectrometrically. The latter method provided higher absorption than the single extraction. Comparing of the analysis time of double extraction with the other methods, this procedure provided long analysis time. Nevertheless, the method can be recommended as an alternative when using for trace determination of tetracycline hydrochloride in samples, which provides better sensitivity than the single one.

3.3.5. Sample volume

The volume ratio between aqueous to organic phases was studied. The preconcentration efficiency attained is directly controlled by adjusting the aqueous/organic ratios in the extraction steps. Increasing sample volume, the sensitivity was found to be increased.

3.4. Analytical characteristics

Following the proposed SIA lab-at-valve system under the sets of the selected conditions, a linear calibration graph was obtained for tetracycline hydrochloride determination in the concentration range of 80-280 ppm with the calibration equation: $y = 0.0018x + 0.0087$, $R^2 = 0.9922$; the LOD being 4.3 ppm. The relative standard deviation (%RSD) was less than 3.3 (n = 11, 120 ppm tetracycline hydrochloride). Sample throughput of 8 samples per hour was achieved. The consumption of sample and organic solvent in one analytical cycle was 400 μL and 200 μL, respectively.

3.5. Application to samples

Under the proposed working conditions, the SI lab-at-valve system was applied to the determination of tetracycline hydrochloride in some local commercial pharmaceutical preparations taken as samples to be assayed. A weighed quantity of sample was dissolved in deionized water; the solutions were shaken in an ultrasonic bath for 5 minutes and adjusted to a volume with water to obtain a solution having a concentration in the range of a calibration graph. The results for determination of tetracycline hydrochloride in some pharmaceutical preparation samples and % recovery of the extraction procedure are listed in Table 2.

Table 2 Determination of tetracycline hydrochloride in some pharmaceutical preparation samples and % recovery

Sample	Label amount (mg)	Amount found (mg)	% Label*	%Recovery**
Sample 1	250	272	109	107
Sample 2	250	263	105	104
Sample 3	250	273	109	100

*% Label is estimated by (Amount found) x100/ (Label amount)
**%Recovery is estimated by (Found value-added standard) x100/added standard

4. Conclusion

Sequential injection lab-at-valve (SI-LAV) for micro-solvent extraction assay of tetracycline based on ion association was investigated. It was applied to the assay of tetracycline hydrochloride in pharmaceutical samples. The procedure is automated and leads to micro-volume analysis. It should be useful for extraction of the tetracycline antibiotics in various kinds of samples, including biological fluids, of which the amounts are limited for analysis and it could be an alternative, especially for places where the expensive equipment is not available. This also serves as green analytical chemistry.

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