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Employing Sequential Injection Analysis Technique and Chemometric Optimization Approach for Developing Diltiazem Assay Method

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ABSTRACT

Sequential injection analysis (SIA) technique and chemometric optimization were employed, for the first time, to develop a new method for the assay of diltiazem in pharmaceutical formulations. The method was based on the spectrophotometric detection of permanganate reduction by diltiazem in acidic media at 526 nm. The 2^3 full factorial design and response surface plot were adopted to optimize permanganate concentration, acid concentration and flow rate. The method was validated as per the International Union of Pure and Applied Chemistry (IUPAC) guidelines. Relatively, wide Beer's law limit (10-180 mg/L) with satisfactory linearity (correlation coefficient = 0.9996) and recovery (95.4-98.1%) were obtained. The method also recorded good repeatability (RSD = 1.19-1.64%, n = 10) and intermediate-precision (2.84%, n = 5, once per a day). The method is sensitive enough for determining diltiazem in pharmaceutical formulations with limits of detection (1.30 mg/L) and quantification (3.94 mg/L). The method is rapid with sample frequency of 32 sample/h. The method was applied to real pharmaceutical samples and the results obtained were realized by parallel analysis by another validated method. The method enjoys the potentials of SIA and chemometric optimization with respect to good accuracy, precision, sensitivity and rapidity. The method is also inexpensive in terms of instrumentation as well as the consumption of reagents and samples. Besides good safety for handling solutions, the SIA technique offers significant waste minimization and manpower reduction to the proposed method. Therefore, the SIA method is suitable in pharmaceutical laboratory for quality control purpose.

Key words: sequential injection analysis, chemometrics, diltiazem, pharmaceutical analysis

INTRODUCTION

In recent pharmaceutical analysis, there is an increasing demand for rapid, instrumentally inexpensive and reagent/sample saving methods. In addition, safety in handling reagents and sample as well as safety to the environment are as important as other analytical aspects. Flow injection (FI) techniques are well-suited to fulfill the requirements of modern pharmaceutical analysis. FI techniques are in continuous development and up-to-date three generations were introduced. The first generation is flow injection analysis (FIA). It was introduced by Ruzicka and Hansen in 1975[1]. In 1990, Ruzicka and Marshall introduced sequential injection analysis (SIA), as the second generation, with dramatic modifications and developments[2]. Thereafter, Ruzicka *et al*. developed the third generation, i.e. bead injection analysis (BIA), with special applications[3]. In spite of the advantages of SIA over FIA, the latter technique is still most dominant. This may be attributed to the fact that FIA is simpler and more familiar than SIA. However, SIA enjoys outstanding features including full-automation, miniaturization and versatility. Publications demonstrating the principles and developments of SIA are available elsewhere[4-7].

Due to its useful potentials, SIA has received special attention in pharmaceutical analysis for different purposes including serial assays, drug dissolution testing and drug screening. The applications of SIA to pharmaceuticals are discussed in a number of informative publications[8-13].

On the other hand, developing new analytical methods requires critical optimization of experimental conditions, which potentially control analysis. Univariate, as the most

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popular optimization approach, optimizes experimental conditions one-by-one by varying levels of one condition while keeping others constant at unspecified levels. However, the chemometrics approach, which is based on multivariate analysis, is more powerful than the univariate approach. Chemometrics gains the highest efficiency of analytical methods in the shortest way. This feature saves time, minimizes effort as well as reduces the consumption of reagents and samples. Chemometrics performs optimization throughout the following means : (i) Examining the main and the interaction effects of experimental conditions on the efficiency of analytical methods; (ii) Optimizing experimental conditions considering their interactions; (iii) Developing simultaneously more than one analytical aspect; (iv) Reducing a large amount of data that can be easily interpreted; (v) Testing the ruggedness. More details on the principles and applications of chemometrics are available elsewhere[14]. Successful applications of exploiting chemometrics for optimizing SIA methods were reported elsewhere[15-23].

Diltiazem hydrochloride, which belongs to benzothiazepine class, is chemically named 3-acetyloxy-5-(2-(dimethylamino)ethyl)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)one monohydrochloride (Figure 1). Diltiazem is a calcium channel blocker widely used as an anti-anginal and anti-hypertensive agent. Due to its popularity, several methods for the assay of diltiazem have been reported. The United States Pharmacopoeia (USP) specifies a non-aqueous potentiometric titration method in pure and tablet form[24]. The British Pharmacopoeia also specifies potentiometric titration method for bulk form while no method for pharmaceutical formulations is described[27]. In addition, several spectrophotometric[26-37], high performance liquid chromatographic[38], micellar liquid chromatographic[39,40] and high-performance thin-layer chromatographic[41,42] methods were reported.

The current study deals with utilizing SIA technique with chemometric optimization approach for developing a new assay method for diltiazem in pharmaceutical formulations. The method is based on the oxidation of the drug by permanganate in acidic media. The reduction of permanganate was spectrophotometrically detected. Permanganate, as a superior oxidizing agent with high absorptivity, was found to be selective in highly controlled experimental conditions[19,43,44].

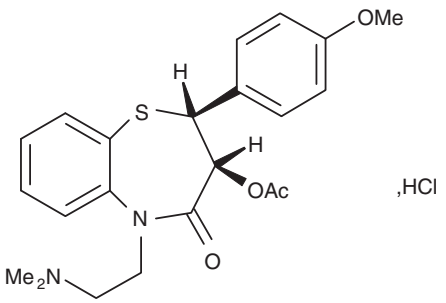

Figure 1. Chemical structure of diltiazem hydrochloride

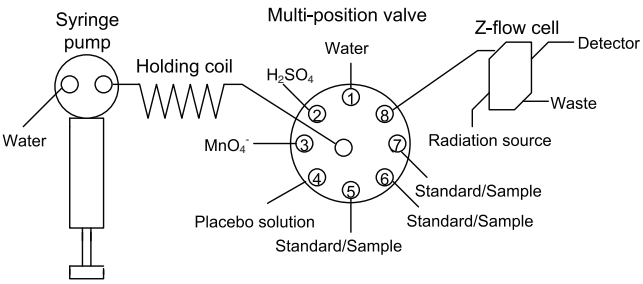

Figure 2. Schematic diagram of a SIA manifold constructed for diltiazem assay

MATERIALS AND METHODS

I. *Instrumentation and Software Packages*

The manifold used in this work is composed of a SIA system combined with a miniaturized fiber optic spectrometer. The components of the manifold are diagrammed in Figure 2.

The SIA system is FIALab 3500 (Medina, WA, USA). It is composed of a syringe pump (SP), multi-position valve (MPV), holding coil (HC) and Z-flow cell (Z) as well as pump tubing and personal computer (PC). The SP includes 24,000 increments with a high-resolution stepper motor, which drives the piston at rates from 1.5 s to 10.0 min per stroke. The SP is > 99% accuracy at full stroke. The syringe has a volume of 2.5 mL. The MPV is chemically inert and has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid) and zero dead volume. The Z is 10 mm path-length Plexiglass compatible with fiber optic connectors. Pump tubing of 0.03" I.D. Teflon type supplied from Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used to connect SIA units and to make a HC (150 cm long).

The optical devices were composed of a radiation source, spectrometer and fiber optic connectors. They were fabricated by Ocean Optics (Dunedin, FL, USA). The radiation source is an LS-1 Tungsten Halogen lamb optimized for VIS-NIR (360 nm-2 μm wavelength range). The detector is a USB2000 Spectrometer adapted to 200-1100 nm wavelength range. The fiber optic connectors are 200 micron SubMiniature version A (SMA).

II. *Software Packages*

FIALab® for Windows version 5.0 supplied from FIAlab (Medina, WA, USA) was used for programming and controlling SIA manifold. Spectrophotometric data was collected and processed using OOIBase® Software version 2.0.1.2 supplied from Ocean Optic, Inc

(Dunedin, FL, USA). SigmaPlot® for Windows version 9.01 supplied from Systat Software, Inc. (Point Richmond, CA, USA) was used for interpolating data and constructing surface plots.

III. *Chemicals and Reagents*

All chemicals and reagents used in this study were of analytical reagent grade. The quality of water was distilledly deionized. Diltiazem hydrochloride as standard material was supplied from Sigma-Aldrich (Quımica S.A., Spain). Potassium permanganate and sulfuric acid were supplied from Fluka (Buchs, Switzerland). Some inactive ingredients possibly found in tablet and capsule formulations were a generous gift from Salah Factory (Khartoum North, Sudan). Inactive ingredients included sodium citrate, microcrystalline cellulose, magnesium stearate, maize starch, titanium dioxide, carnauba wax, propylene glycol, povidone and talc.

V. *Pharmaceutical Samples*

Adizem-SR® tablets (120 mg diltiazem) and Adizem-XL® (200 mg diltiazem) capsules manufactured by Napp Pharmaceuticals Ltd. (Cambridge, UK) were examined in the current study. Tildiem® tablets (60 mg diltiazem) manufactured by Sanofi-aventis Ltd. (Surrey, UK) as well as Dilzem-XL® capsules (180 mg diltiazem) manufactured by Cephalon Ltd. (Hertfordshire, UK) were also examined.

VI. *Preparation of Reagents and Standard Solutions*

A stock standard solution of 1000 mg/L diltiazem was prepared by dissolving an appropriate amount directly in water. In addition, a stock standard solution of 0.02 M potassium permanganate was prepared and standardized weekly. Working standard solutions of diltiazem, permanganate and sulfuric acid were prepared by dilution in an appropriate way.

IIIV. *Preparation of Pharmaceutical Samples*

Ten tablets were ground and 10 capsules were refilled. An equivalent amount of 10 mg diltiazem powders of tablets and capsules were accurately weighed. The weights were extracted into 25 mL chloroform and filtrate through Whatman® No. 42 filter paper. The filtrate was evaporated under vacuum to dryness. The residue was resuspended with distilled deionized water and transferred to a 100 mL volumetric flask and diluted to volume.

Three placebo samples were prepared including inactive ingredients in a concentration range of 20-100 mg/L

VII. *SIA Procedure*

As shown in figure 2, a single-channel SIA manifold was constructed to perform on-line developing reaction and spectrophotometric measurement. Port-1 in the MPV was linked with the Z. Water was linked with both the SP and port-2 in the MPV. Permanganate solution was linked with port-3. Standards/samples were attached to ports-3-8. A rapid protocol controlling the proposed SIA procedure was programmed. It is briefly described as follows.

i. Following the practice of SIA, the syringe was firstly filled with 1500 µL of water for propelling solutions.
ii. Each solution was loaded into the HC by aspiration using the SP. The excess of solutions was dispensed to the waste.
iii. The syringe was filled again with 1500 µL of water.
iv. 30 µL of each of sulfuric acid and permanganate solutions were sequentially aspirated into the HC.
v. The solutions were mixed by reverse-flow of 10 µL at a flow rate of 30 µL/s.
vi. 50 µL of water, as a blank solution, was injected into the HC and mixed.
vii. The mixture was dispensed through the Z at the required flow rate. During flowing, the maximum permanganate absorbance was recorded at 570 nm.
viii. For standard/sample measurement, steps iii-vii were repeated with replacing standard/sample instead of blank.
ix. The response «R» of permanganate reduction was calculated as the difference of absorbance before and after adding standard/sample.

RESULTS AND DISCUSSION

I. *Chemometric Optimization*

Primarily, it has been found that diltiazem is possibly oxidized by permanganate in sulfuric acidic media. Spectrophotometric study showed that there was no spectrum interference at 526 nm, a wavelength that was set for detecting permanganate absorbance before and after reduction. Suitable ranges of permanganate concentration, acid concentration and flow rate were examined for chemometric optimization. At permanganate concentration more than 5.0×10^{-3} M, lower diltiazem concentration in samples may record insignificant reduction of permanganate absorbance. On the other side, permanganate concentration less than 5.0×10^{-4} M may narrow the Beer's law limit. For sulfuric acid concentration, the range of 1.0×10^{-2}-1.0×10^{-3} M recorded, primarily, significant reduction of permanganate by diltiazem. In general, higher sulfuric acid concentration distorts the base line of the SIAgram and produces non-repeatable spectrophotometric measurement[19,20,22,23,44].

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Table 1. 2^3 full factorial design matrix with experimental results (responses)

Experiment number	Permanganate concentration (M)	Sulfuric acid concentration (M)	Flow rate (μL/s)	Response
1	5.0×10^{-4}	1.0×10^{-3}	10	0.136
2	5.0×10^{-4}	1.0×10^{-3}	40	0.172
3	5.0×10^{-4}	1.0×10^{-2}	10	0.108
4	5.0×10^{-4}	1.0×10^{-2}	40	0.160
5	5.0×10^{-3}	1.0×10^{-3}	10	0.255
6	5.0×10^{-3}	1.0×10^{-3}	40	0.297
7	5.0×10^{-3}	1.0×10^{-2}	10	0.222
8	5.0×10^{-3}	1.0×10^{-2}	40	0.264

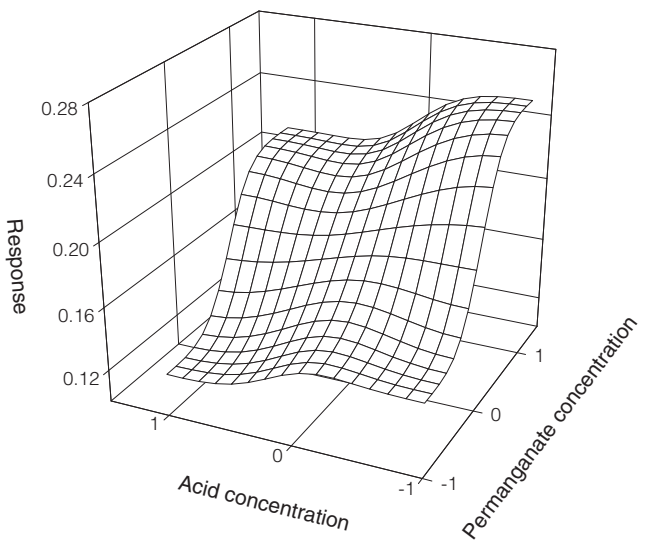

Figure 3. Response surface plot of permanganate concentration (M) against acid concentration (M)

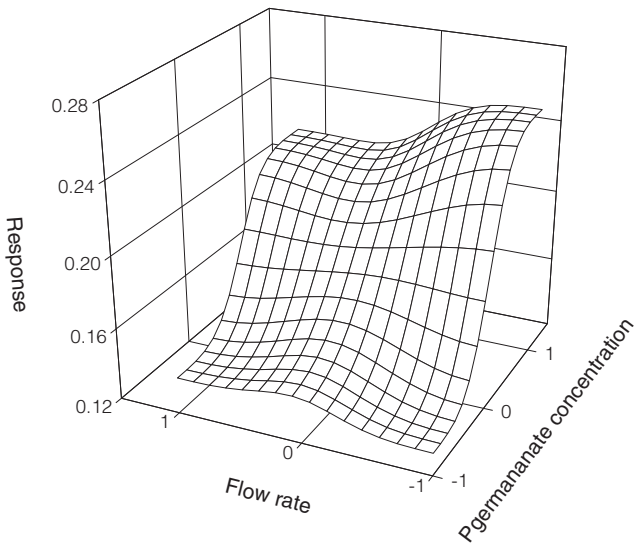

Figure 4. Response surface plot of permanganate concentration (M) against flow rate (μL/s)

Regarding flow rate for spectrophotometric measurement, following the practice of SIA, a range of 10-40 μL/s is always suitable.

The 2^3 full factorial design was adopted in the current study. The base 2 stands for the number of experimental conditions levels. The power 3 stands for the number of experimental conditions would be optimized. A total of 8 experiments were carried out using experimental conditions as illustrated in Table 1. The results obtained are also introduced in Table 1.

The data in the adopted factorial design matrix including response values was interpolated using values (-1) and (+1) instead of the minimum and the maximum values of experimental condition levels[7]. Then, the response surface was plotted and depicted in Figures 3-5.

Figure 3 shows a positive permanganate concentration effect on response while a negative acid concentration effect on response is recorded. Moreover, the order of permanganate concentration effect is higher than that of acid concentration effect. In general, high permanganate concentration enhances the oxidation of diltiazem. Negative acid concentration effect may be due to relatively low acidity of diltiazem. On the other hand, the trend of acid concentration effect on response, which is negative (Figure 3), is similar to the trend of flow rate effect (Figure 4). Negative effect of flow rate indicates that the oxidation of diltiazem by permanganate is slow. Therefore, the reaction could be enhanced by applying low flow rate, which allows reaction to take place before measurement. Figure 5 shows that the order of flow rate effect on response is higher than that of acid concentration effect. In Figure 3, the semi-flat surface appeared

in the corner of higher permanganate concentration and lower acid concentration indicates that there is no need to examine further levels beyond levels adopted in the current study. The outputs of both Figures 4 and 5 strengthen this finding. On the other hand, Figures 4 and 5 also depict semi-flat surface with respect to flow rate. Hence, a flow rate of 15 μL/s was examined and similar response was recorded. Eventually, the finding of the chemometric optimization is that 5.0×10^{-3} M permanganate, 1.0×10^{-2} M sulfuric acid and 15 μL/s flow rate were set as the optimum levels.

II. *Method Validation*

Under the optimized experimental conditions, the SIA method was validated based upon the International Union of Pure and Applied Chemistry (IUPAC) guidelines[45] in terms of Beer' law limit, recovery, repeatability, intermediate-precision and sample frequency as well as limits of detection and quantification.

To examine the Beer' law limit, a long series of standard solutions of diltiazem (1-300 mg/L) was subjected to the proposed SIA method. The method was found to be linear in the range of 10-180 mg/L. The weighed regression of the calibration equation obtained was "R = 0.0025C + 0.0368", where R is the response (absorbance unit) and C is diltiazem concentration in mg/L. The correlation coefficient obtained was 0.9996 indicating good linearity.

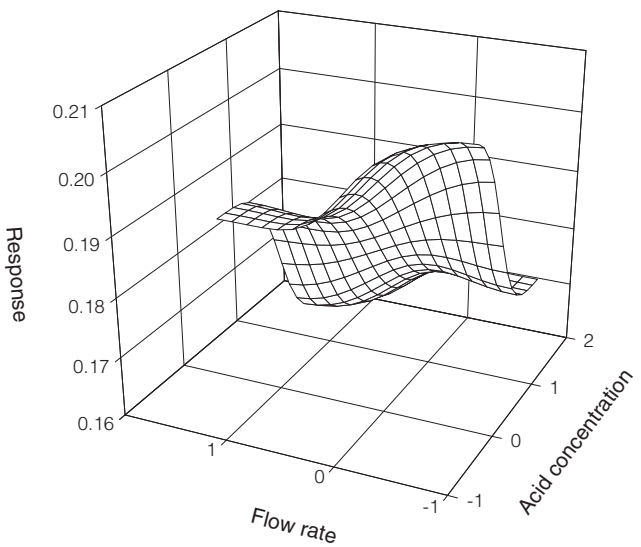

Figure 5. Response surface plot of acid concentration (M) against flow rate (μL/s)

Three placebo samples were subjected to the SIA method. Each sample was analyzed three times for diltiazem. No reduction of permanganate under the optimum conditions was recorded indicating good selectivity of the SIA method in the presence of excipients usually found in tablet and capsule formulations.

The intermediate-precision was also examined by replicating 5 experiments, each per day within one week. The RSD was found to be 2.84% indicating also satisfactory intermediate-precision. Sample frequency of the proposed method was calculated. The software controlling the SIA system counted 112.5 s for each experiment. Therefore, sample frequency obtained was 32 sample/h, which indicates fast analysis.

The limit of detection (LOD) was examined as the concentration of solute resulting in a peak height three times the baseline noise level while the limit of quantification (LOQ) was examined as the concentration of solute resulting in a peak height ten times the baseline noise level. The LOD and LOQ were 1.30 and 3.94 mg/L, respectively.

III. *Application*

Pharmaceutical samples were subjected to the validated SIA method. The same tablet samples were also subjected to the USP method while the same capsules samples were subjected to a spectrophotometric method, which was based on the formation of colored chloroform extractable ion-pair complex of the diltiazem with bromothymol blue in acidic medium[32]. In that method, validation study was conducted and acceptable results were obtained.

Samples were analyzed ten times. The repeatability as RSD, mean recovery and t-test value were calculated.

Table 2. Results obtained by the SIA, USP and spectrophotometric methods for the assay of diltiazem in pharmaceutical formulations

Pharmaceutical sample	Formulation and content	SIA method	USP method	Spect.[1] method	t[2]
		M.R.[3] ± RSD(%)[4]	M.R.[3] ± RSD(%)	M.R.[3] ± RSD(%)	
Tildiem-SR®	Tablets (60 mg)	96.5 ± 1.24	94.2 ± 2.70	-	2.08
Adizem-SR®	Tablets (120 mg)	95.4 ± 1.37	95.7 ± 2.51	-	1.98
Dilzem XL®	Capsules (180 mg)	98.1 ± 1.19	-	94.3 ± 3.41	2.31
Adizem-XL®	Capsules (200 mg)	97.9 ± 1.64	-	94.0 ± 3.68	2.09

1: Spectrophotometric; 2: Student t-test values; 3 Mean recovery; 4: Relative standard deviation for 10 replicates

The results obtained are introduced in Table 2. The values of RSD and mean recovery indicate acceptable accuracy and repeatability. The calculated t-test values were found to be lower than that tabulated emphasizing the reliability of the results obtained by the SIA method.

CONCLUSIONS

For the first time, a SIA method for diltiazem assay in pharmaceutical formulations was developed. The method enjoys the potentials of SIA with respect to automation and miniaturization. Comparing with previous methods, the newly developed SIA method is inexpensive with respect to instrumentation as well as reagent and sample consumption. The method is also rapid, accurate and repeatable as well as safe in handling reagents and safe to the environment.

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