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Rapid inexpensive assay method for verapamil by spectrophotometric sequential injection analysis

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Sequential injection analysis (SIA) technique with a miniaturized fibre optic spectrophotometry was exploited to optimize and validate a new method for the assay of verapamil in pharmaceutical formulations. The reduction of acidified permanganate by verapamil was spectrophotometrically detected at 546 nm. The 2^3 full-factorial design was adopted for screening the effect of conditions controlling the proposed method, and accordingly for the purpose of optimization. The remarkable advantages of the method are high rapidity (sample frequency was 10.6 samples/h), saving in reagents and sample (total consumed volume was 190 μl) and better safety for the environment (total waste production volume was 2140 μl). Additionally, the method was selective in the presence of excipients usually found in tablet and injection formulations. The average of recovery in synthetic samples as well as dosage forms was 98.8–103.0%. The obtained results were realized by the British Pharmacopoeia method and comparable results were obtained. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: sequential injection analysis; chemometrics; verapamil; pharmaceutical analysis

Introduction

Because it provides potential economic benefits, spectrophotometry has been extensively applied to various analytical fields, especially pharmaceutical analysis. However, conventional spectrophotometry cannot always meet the requirements of modern industrial-scale pharmaceutical analysis unless automated and miniaturized tools are involved.

Flow injection (FI) techniques have been well-established for modern chemical analysis. Despite the family of FI techniques includes three generations and many versions,[1,2] the second generation sequential injection analysis (SIA)[3] is still the most effective technique. SIA gathers unique features including automation, miniaturization and versatility.

Another challenge in developing effective analytical methods is the optimization process. Univariate, as the most popular optimization approach, optimizes experimental conditions one-by-one by varying levels of one condition while keeping others constant at unspecified levels. However, chemometrics, which is based on multivariate analysis, is more powerful than the univariate approach. Chemometrics offers high efficiency for analytical methods in a short way. Chemometrices also saves time, minimizes effort as well as reduces the consumption of reagents and samples. Chemometrics performs optimization throughout the following means: (1) examining the main and the interaction effects of experimental conditions on the efficiency of analytical methods; (2) optimizing experimental conditions with considering their interactions; (3) developing simultaneously more than one analytical aspect, for example, sensitivity, rapidity, resolution, etc.; (4) reducing a large amount of data that can be easily interpreted; and (5) testing the ruggedness. More details on this issue are available elsewhere.[4]

Verapamil hydrochloride is chemically named 2-(3, 4-dimethoxyphenyl)-5-[{2-(3, 4-dimethoxyphenyl) ethyl} (methyl) amino]-2-(1-methylethyl)pentanenitrile hydrochloride (Figure 1). It is the first clinically useful member of the calcium channel-blocking group. It acts as anti-anginal and anti-arrhythmic. Verapamil slows down conduction through the atrioventricular node and thus slows the increased ventricular response rate that occurs in atrial fibrillation and flutter. In addition, verapamil is sometimes used for the management of myocardial infarction. Verapamil hydrochloride is usually available as a mono component in injection and tablet forms.[5,6]

Due to its importance and worldwide use, various analytical techniques were utilized for the development of many methods for the assay of verapamil in pharmaceutical formulations. In this issue, conventional spectrophotometry,[6–12] fluorometry,[13–15] atomic emission spectrometry,[16] high performance thin-layer chromatography,[17] high performance liquid chromatography,[7,18–25] capillary electrophoresis,[26] and electrochemical methods[27–29] were all utilized. Furthermore, the first generation of FI techniques, i.e. flow injection analysis, with amperometry[30] and conductimetry[31] were exploited. However, in the literature, SIA has not been utilized yet for the development of a method for the assay of verapamil.

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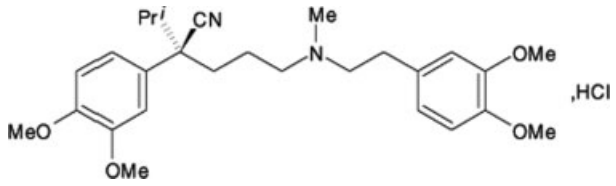

Figure 1. Chemical structure of verapamil hydrochloride.

The current communication describes a chemometric optimization and validation of a new method for the assay of verapamil in pharmaceutical formulations. Our main objective is to utilize the benefits of SIA for developing an analytical procedure enjoying high rapidity, and high safety for the environment and for handling reagents, besides being inexpensive in reagent consumption.

Experimental

Instrumentation

In the current study, the following devices were assembled to construct a SIA system (Figure 2). Unless otherwise prescribed, all devices constructing the SIA system were supplied from FIALab® (Medina, WA, USA).

i. Syringe pump (SP), with a volume of 2.5 ml, includes 24 000 increments with high-resolution stepper motor. The SP drives the piston at rates from 1.5 s to 10.0 min per stroke with >99% accuracy at a full stroke.
ii. Multiposition valve (MPV) – chemically inert and has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid) and zero dead volume.
iii. Z-flow cell (Z) with a 10 mm path-length Plexiglass compatible with fibre optic connectors.
iv. Pump tubes of '0.03 inch' ID Teflon type – used to connect various devices of the SIA system and to make a holding coil (HC) and a reaction coil (RC) with a length of 160 cm for each. Pump tubes were supplied from Upchurch Scientific, Inc. (Oak Harbor, WA, USA).
v. An LS-1 tungsten halogen lamp was manufactured by Ocean Optics (Dunedin, FL, USA). It was optimized for Vis-NIR (360 nm–2 µm wavelength range).
vi. A USB2000 spectrometer was manufactured by Ocean Optics (Dunedin, FL, USA), adapted to 200–1100 nm wavelength range.
vii. Fibre optic connectors were fabricated by Ceram Optec (East Longmeadow, MA, USA). They are 200 micron sub-miniature version A®.
viii. PC equipped with the Fialab® software was used for controlling the SIA system.

Chemicals, reagent, and samples

Distilled deionized water was used in all experiments. Chemicals and reagents were of analytical reagent grade. Verapamil hydrochloride was supplied from Sigma-Aldrich (Taufkirchen, Germany). Potassium permanganate and sulfuric acid (95–98% (w/v), 1.84 g ml^{-1}) were supplied from Fisher Scientific Co. (Fair Lawn, NJ, USA).

Manidon® injection (5 mg/2 ml verapamil) was prepared by Abbott Laboratories S.A. (Madrid, Spain). Isoptin® tablets (40 mg verapamil) were prepared by Abbott GmbH & Co. KG (Ludwigshafen, Germany). Some excipients usually found in tablet and injection formulations, which included sodium citrate, microcrystalline cellulose, magnesium stearate, maize starch, carnauba wax, povidone and talc, were a generous gift from Salah Factory (Khartoum North, Sudan).

Software packages

FIALab® version 5.5 from FIALab® (Medina, WA, USA) was used for programming algorithms that control the SIA system. OOIbase® version 2.0.1.2 from Ocean Optic, Inc. (Dunedin, FL, USA) was used for data acquisition. SigmaPlot® version 1.02 from Jandel Sceintific Corporation (Point Richmond, CA, USA) was used for interpolating data and constructing response surface plots.

Preparation of reagents and samples

A stock standard solution of 1000 µg ml^{-1} verapamil was prepared by dissolving an appropriate amount directly in water. Working standard solutions were prepared daily by dilution in an appropriate way. 20 mmol l^{-1} potassium permanganate was prepared as a stock standard solution. Working standard solutions of permanganate were prepared daily and standardized in appropriate ways. Working solutions of sulfuric acid were prepared by dilution from the primary material.

Twenty Isoptin® tablets (40 mg verapamil) were weighed and ground into a fine powder. An accurately weighed powder equivalent to 50 mg verapamil was transferred into a 50-ml

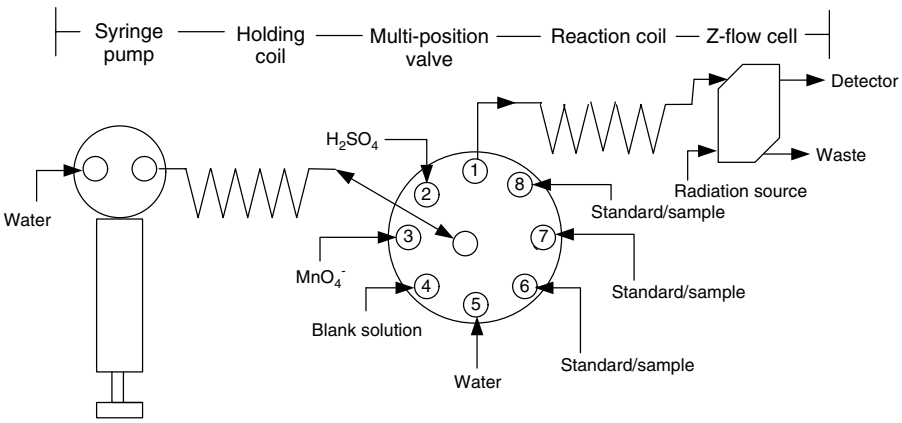

Figure 2. Schematic diagram of a SIA manifold constructed for verapamil assay.

Table 1. The typical sequence of the particular steps of the SIA program controlling the proposed procedure for the assay of verapamil (a^{-1} single circle)

Step #	Action description	Time (s)	Valve position	Pump action	Flow rate (µl s)	Volume (µl)
1	Filling syringe with water	6.7	–	Aspirate	150	1000
2	Loading sulfuric acid	0.6	2	Aspirate	50	30
3	Loading permanganate	0.6	3	Aspirate	50	30
4	Propelling solutions afterward and backward twice for mixing	4	1	Dispense & aspirate	10	10
5	Loading a placebo solution	0.6	4	Aspirate	50	30
6	Propelling solutions afterward and backward twice for mixing	4	1	Dispense & aspirate	10	10
7	Propelling solutions to the RC	2	1	Dispense	150	300
8	Delay solutions in the RC	120	1	–	–	–
9	Simultaneously: propelling solutions to Z spectrometer reference scan spectrometer absorbance scan	32	1	Dispense	25	800
10	Repeat steps # 1–4	11.9				1070
11	Loading standard/sample solution	0.6	4	Aspirate	50	30
12	Repeat steps # 6–9	158				1110
Total		341	–	–	–	2140

calibrated flask. Water was added to extract verapamil and the obtained solution was thoroughly shaken for 30 min. The solution was diluted with water to the mark, mixed well and filtered using a quantitative Whatmann® filter paper No. 42. An aliquot volume was diluted appropriately to obtain working solutions.

For injection formulation, five ampoules of Manidon® injection (5 mg/2 ml verapamil) were dispensed and homogenized. An appropriate portion of sample was transferred into a 25-ml volumetric flask to obtain 50 µg ml^{-1} verapamil. Both tablets and injection samples were subjected to the proposed SIA method in triplicate.

A placebo pharmaceutical sample was synthesized in triplicate at our laboratory. A placebo sample included excipients in a total concentration of 30 µg ml^{-1}. Furthermore, synthetic pharmaceutical samples were prepared in triplicate in our laboratory. The latter sample included 50 µg ml^{-1} verapamil as well as excipients in a total concentration of 30 µg ml^{-1}.

SIA procedure

As shown in Figure 2, sulfuric acid, permanganate, blank solution, and water (as a spacer solution) were linked with port-2 to -5, respectively. Verapamil standard/sample solutions were linked with port-6 to 8. HC was installed between the central port in the MPV and the 'In' position in the SP. Water (as a propeller liquid) was linked with the 'Out' position in the SP. RC and Z with spectrometric devices were attached with port-1. A rapid protocol controlling the proposed SIA procedure was programmed as illustrated in Table 1.

Results and discussion

Method optimization

Preliminary study

Primarily, it has been found that verapamil is oxidized by permanganate in sulfuric acid media in a slow reaction. The absorbance of acidified permanganate was detected before and after adding verapamil. The reduction in absorbance was considered as the parameter of the optimization process. Hence, the main target of the optimization process is that to maximize that parameter as much as possible. This development could improve the limit of detection and the limit of Beer's Law.

For wavelength optimization, it is well known that permanganate has several wavelengths recording high absorbance, i.e. 507, 526, and 546 nm. Despite that $\lambda_{max\,1}$ is 526 nm, 546 nm was set as the optimum because it showed more convenient absorbance values with respect to the adopted permanganate concentration.

Experimental design optimization

When applying experimental design approaches, it is advisable to keep the number of variables as low as possible. That trend avoids complex response models and large variability.[4] It has been reported that in previous spectrophotometric SIA methods,[32–41] reagent concentration has a strong impact on the efficiency of those methods. Therefore, the concentrations of permanganate and sulfuric were considered for optimization in the current study. Furthermore, it has been found that the adopted reaction was slow. Hence, to complete the reaction, the condition of a delay time in the RC was considered for optimization as well. On the other hand, following the practice of SIA,[32–34] flow rate was fixed at 25 µl s^{-1}.

The 2^3 full-factorial design was adopted. The base 2 stands for the levels of experimental conditions. The power 3 stands for the number of experimental conditions. The minimum and maximum practicable levels of conditions are introduced in Table 2.

At permanganate concentration higher than 1.0 mmol l^{-1}, low verapamil concentration may not record significant reduction of permanganate. At permanganate concentration lower than 2.0 mmol l^{-1}, a wide limit of Beer's Law might not be obtained. For sulfuric acid concentration, the range of 1.0–10.0 mmol l^{-1} recorded, primarily, significant reduction of permanganate. In general, high sulfuric acid concentration distorts the base line of the SIA-gram and produces non-repeatable spectrophotometric measurement.[32–37,39–41] The preliminary study revealed that

Table 2. Low and high levels of acid concentration, permanganate concentration and delay time applied to the 2^3 full-factorial design optimization

Experimental condition	Low level	High level
Acid concentration (mmol l^{-1})	1.0	10.0
Permanganate concentration (mmol l^{-1})	1.0	2.0
Delay time (min)	2.0	4.0

Table 3. 2^3 full-factorial design matrix for the optimization of SIA method for the assay of verapamil

Experiment #	H_2SO_4	$KMnO_4$	Time	Response
1	−1	−1	−1	0.101
2	−1	−1	+1	0.118
3	−1	+1	−1	0.176
4	−1	+1	+1	0.216
5	+1	−1	−1	0.146
6	+1	−1	+1	0.162
7	+1	+1	−1	0.244
8	+1	+1	+1	0.240

2.0–4.0 min has been found to be a suitable range of delay time, which a complete reaction could be obtained.

Using the consequence that introduced in Table 3, a total of eight experiments were conducted. Each set of conditions was replicated three times. Because of the full-automation of the developed SIA procedure, the relative standard deviation (RSD) values for each set did not exceed 1.0%.

Response surface approach

The coded levels that are introduced in Table 3 were interpolated using the SigmaPlot® software package. The response surface plots for each two conditions were constructed. As shown in Figures 3A and 3B, in general, the response increases as permanganate concentration and acid concentration increase, but to a lesser extent. This means that the effect of permanganate concentration is higher than that of acid concentration. In more detail, comparing Figure 3A with Figure 3B, the effect of permanganate concentration at a short delay time is lower than that at a long delay time and vice versa for acid concentration. On the other hand, almost the same response was recorded at the conditions of high permanganate concentration and long delay time with either low or high acid concentration (Figures 4A and 4B). In Figures 5A and 5B, a slight effect was recorded at high acid concentration with a short or a long delay time. Furthermore, significant development in the response was achieved when applying conditions as in Figure 5B.

Effect factor

The main effect factor of each condition and the interaction effect factor between conditions were calculated using Equation 1.[4] $y(+1)$ and $y(-1)$ are the response values at the maximum and the minimum levels of an examined condition, respectively. n is the number of variables at the same level. In the current case, $n = 4$. The obtained results are depicted in Figure 6. It

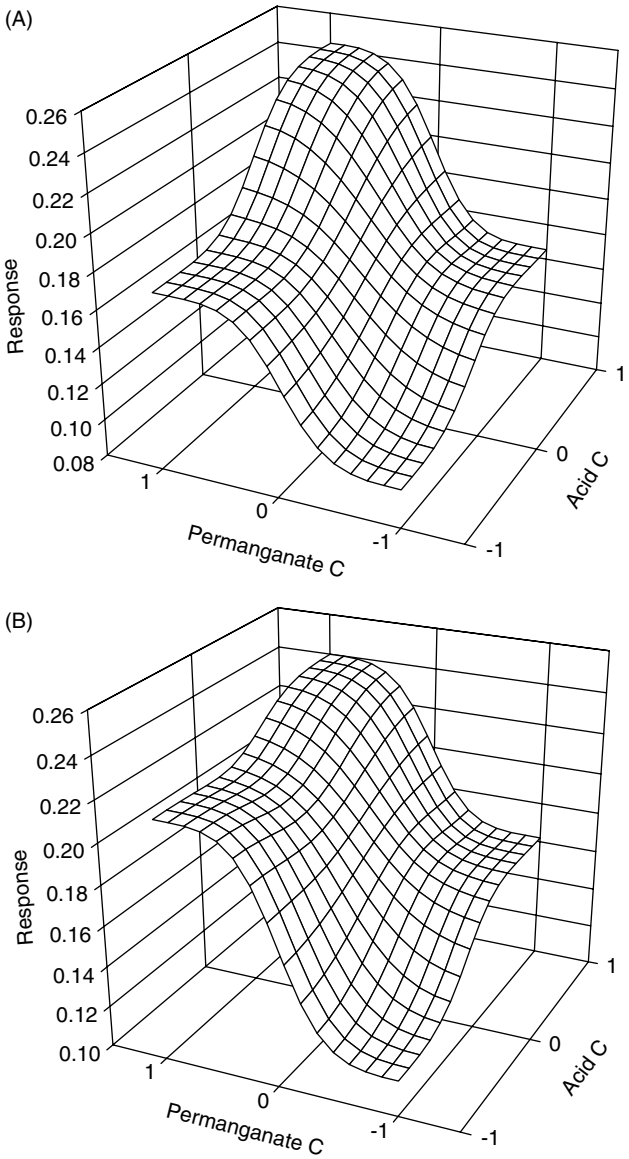

Figure 3. Response surface plots of acid concentration against permanganate concentration using delay time of: (A) 2.0 min and (B) 4.0 min.

has been found that the order of effect of the main factors is as follows: permanganate concentration > acid concentration > delay time. All those factors positively affected response. Moreover, less negative effect was recorded for the interaction between acid concentration and delay time as well as for the interaction between the three examined conditions. Furthermore, insignificant positive effect was recorded from the interaction between permanganate concentration and acid concentration. The same type and level of effect were also recorded from the interaction between permanganate concentration and delay time. These findings strengthen what is explained by the surface plot, i.e. that permanganate concentration and delay time interacted with each other more than their interaction with acid concentration.

$$E_f = \frac{\sum y(+1)}{n} - \frac{\sum y(-1)}{n} \quad (1)$$

In conclusion, the maximum concentration of permanganate, i.e. 2.0 mmol l^{-1}, was set as the optimum because potential

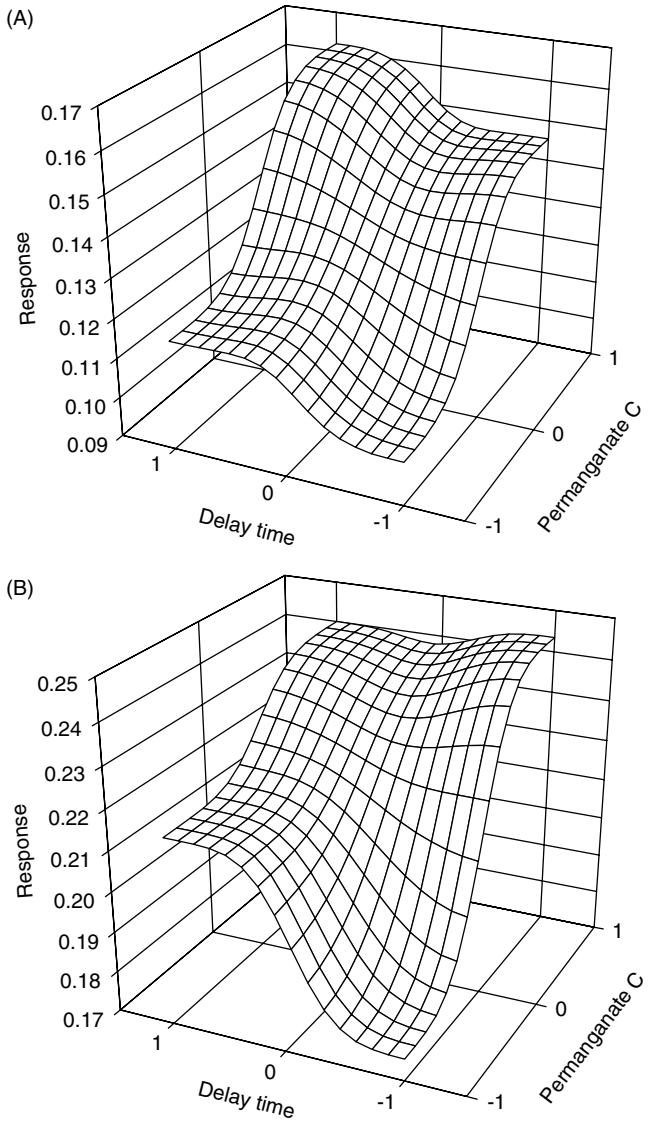

Figure 4. Response surface plots of permanganate concentration (C) against delay time using acid concentrations of: (A) 1.0 mmol l^{-1} and (B) 10.0 mmol l^{-1}.

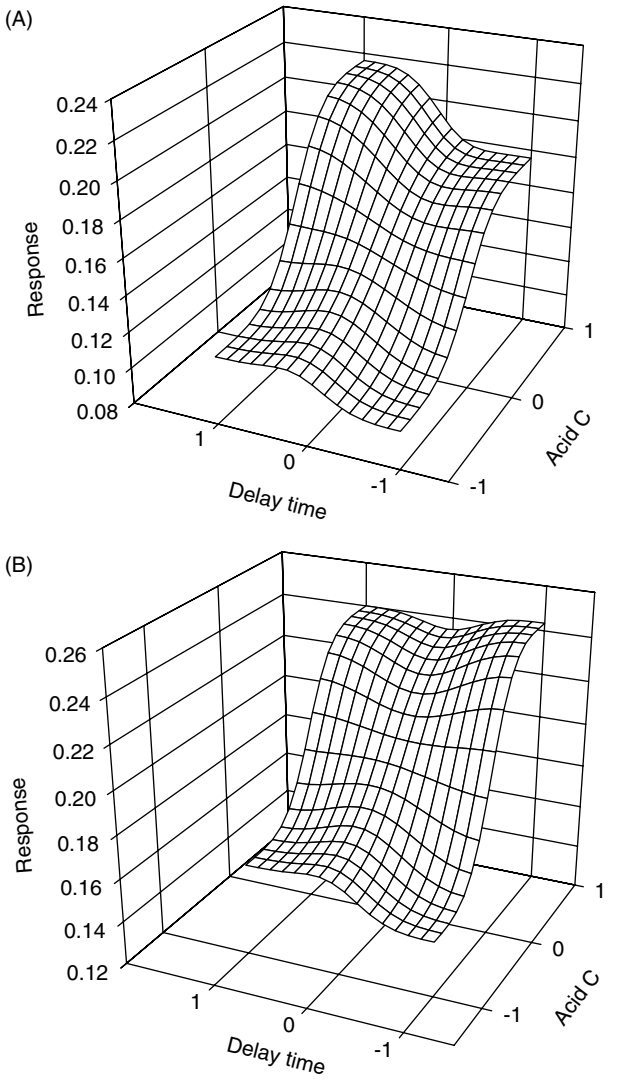

Figure 5. Response surface plots of acid concentration against delay time using permanganate concentration of: (A) 1.0 mmol l^{-1} and (B) 2.0 mmol l^{-1}.

improvement in response has been obtained at that level. Despite acid concentration positively affecting the response, but not significantly, the minimum concentration 1.0 mmol l^{-1} was set as the optimum. The use of low acid concentration enhances the repeatability of SIA[32–37,39–41] and provides better safety for the environment. On the other hand, no significant improvement in response at a long delay time could be observed. Hence, 2 min was set as the optimum.

Method validation and application

The proposed SIA method was validated according to the guidelines of the main regulatory agencies, namely International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use[42] and the International Union of Pure and Applied Chemistry (IUPAC).[43] The results obtained are summarized in Table 4.

Linearity

Several standard solutions of verapamil, namely 10, 25, 50, 100, 200, 300, 400, and 500 μg ml^{-1}, were subjected to the proposed SIA method. By the virtue of the optimization process, a wide range (50–200 μg ml^{-1}) with satisfactorily linearity (R = 0.9999) was obtained.

Precision

The intra- and inter-day precision of the proposed SIA method were examined. The intra-day precision was evaluated by preparing and analyzing 21 synthetic pharmaceutical samples including three concentrations of verapamil, namely 50, 100, and 200 μg ml^{-1}; each concentration was prepared seven times. Samples were then sequentially subjected to the proposed SIA procedure. Similarly, the inter-day precision was evaluated by preparing and analyzing the same synthetic samples on five consecutive days. Verapamil concentrations were determined and the RSD values were calculated. In general, as shown in Table 4, the full-

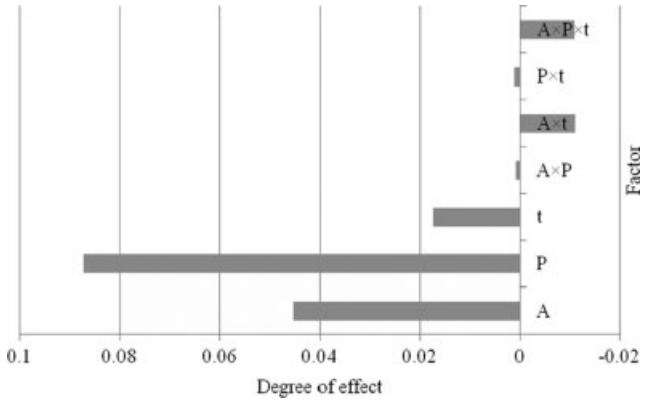

Figure 6. The main and interaction effect of acid concentration (A), permanganate concentration (P) and delay time (t), besides their interaction, on the response of the verapamil assay method utitlizing spectrophototmtey sequential injection analysis.

Table 4. Validation results of the SIA method for the assay of verapamil

Analytical aspect	Value
Weight regression of calibration equation	$R^a = 0.001C + 0.047$
Correlation coefficient	0.9999
Limit of the Beer's law (μg ml^{-1})	50–200
Intra-day precision (RSD%, n = 7)	2.08
Inter-day precision (RSD%, n = 5)	3.13
Limit of detection (μg ml^{-1})	0.80
Limit of quantification (μg ml^{-1})	2.60
Sample frequency (samples s^{-1})	10.6
Total reagent and sample consumed (μl)	190
Total waste volume (μl)	2140

[a] response (difference in absorbance of permanganate before and after adding verapamil.

automation of the utilized technique rendered the method highly precise.

Limits of detection and quantification

The limit of detection (LOD) was calculated as the concentration of the solute resulting in a response three times the baseline noise level. The limit of quantification LOQ was calculated as the concentration of the solute resulting in a response ten times the baseline noise level. Table 4 shows satisfactorily LOD and LOQ, which were achieved by the optimization process.

Recovery and application

The proposed SIA method was applied to pharmaceutical formulation samples, namely tablets and injection. The same samples were analyzed in parallel using the British Pharmacopoeia (BP) method.[6] For tablet and injection formulations, the BP recommended a conventional spectrophotometric method at 278 nm. The recovery was calculated using the results obtained from the BP method as reference values. Each sample was analyzed in triplicate using the both SIA and BP methods. The average and RSD values were calculated. The results achieved are summarized in Table 5. The proposed SIA method was accurate and selective

Table 5. Recovery and repeatability of the SIA method for the assay of verapamil in pharmaceutical samples

Verapamil formulation (content)	Recovery[a] %	RSD[b] %	Official range %[c]
Synthetic sample (50 μg ml^{-1})	99.8	1.20	99.0–101.0
Manidon® injection (5 mg/2 mL)	99.0	0.98	90.0–110.0
Isoptin® tablets (40 mg)	102.1	3.01	92.5–107.0

[a] Recovery was calculated using results obtained from the BP method as reference values.
[b] The RSD for three replicates.
[c] The official range of the content of a drug in its formulation as specified by the BP.

since no significant interference was detected from inactive ingredients possibly found in tablets and injection formulations. Additionally, the placebo sample was subjected to the proposed SIA method. No significant reduction in permanganate was recorded. This emphasizes the absence of interference from inactive ingredients.

Other analytical features

It is noteworthy mentioning that the remarkable advantages of the proposed SIA method over other previous methods using various techniques are high rapidity, reagent-saving, and safety for the environment. As illustrated in Table 1, the total analysis time of the proposed SIA procedure, including loading carrier, reagents, and sample, is 341 s. Hence, the sample frequency is 10.6 samples h^{-1}. The miniaturization and full automation of SIA work hand in hand to drastically reduce analysis time. Furthermore, the miniaturization renders the method reagent-saving. The total volume of consumed reagents and sample was 190 μl. On the other hand, the drastic reduction of the consumed volumes also offered better safety for the environment. The total volume of produced waste, including water as a carrier, was 2140 μl.

Conclusions

This paper reports the development of a new assay method for verapamil utilizing spectrophotometric SIA with a chemometric optimization approach. By virtue of the miniaturization and automation of SIA, the proposed method enjoys satisfactory analytical features with respect to accuracy, precision, and rapidity, in addition to safety for handling reagents and safety for the environment. The method is also inexpensive in terms of instrumentation cost, reagent and sample consumption, effort, and manpower. The implementation of chemometric optimization improves the efficiency of the proposed method. Furthermore, the optimization process was carried out in a short time, using minimum effort with less consumption of reagents and samples.

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References

[1] A. M. Idris, *Crit. Rev. Anal. Chem.* **2010**, *40*(3), 150.
[2] A. M. Idris, *Crit. Rev. Anal. Chem.* **2010**, *40*(4), 218.
[3] J. Ruzicka, G. D. Marshall, *Anal. Chim. Acta* **1990**, *237*(2), 329.
[4] E. D. Morgan, *Chemometrics: Experimental Design*, ACOL, John Wiley & Sons Ltd: Chichester, UK, **1991**.
[5] S. C. Sweetman, *Martindale: The Complete Drug Reference*, *35th ed.*, The Pharmaceutical Press: London, **2007**.
[6] *British Pharmacopoeia, 4th ed.*, E-copy on CD, **2009**.
[7] A. Bright, T. S. Renuga Devi, S. Gunasekaran, *Int. J. ChemTech. Res.* **2010**, *2*(2).
[8] N. Rahman, S. N. Hejaz Azmi, *Farmaco* **2004**, *59*(7), 529.
[9] N. Rahman, M. N. Hoda, *Anal. Bioanal. Chem.* **2002**, *374*(3), 484.
[10] W. Caiyun, *Chinese Pharm. J.* **2000**, *35*(1), 45.
[11] A. H. Prabhakar, R. Giridhar, V. B. Patel, *Indian Drugs* **1999**, *36*(6), 372.
[12] N. Erk, *STP Pharma Sci.* **1996**, *6*(5), 349.
[13] J. M. M. Leitao, J. C. G. Esteves Da Silva, A. J. Girón, A. Munoz De La Pena, *J. Fluoresc.* **2008**, *18*(6), 1065.
[14] M. Walash, F. Belal, N. El-Enany, A. Abdelsalam, *J. AOAC Int.* **2006**, *89*(6), 1565.
[15] J. C. G. Esteves Da Silva, J. M. M. Leitão, F. S. Costa, J. L. A. Ribeiro, *Anal. Chim. Acta* **2002**, *453*(1), 105.
[16] S. Khalil, A. Kelzieh, *J. Pharm. Biomed. Anal.* **2002**, *27*(1-2), 123.
[17] D. Kowalczuk, *J. AOAC Int.* **2005**, *88*(5), 1525.
[18] A. Gumieniczek, H. Hopkala, *J. Liq. Chromatogr. R. T.* **2001**, *24*(3), 393.
[19] Y. Ozkan, N. Yilmaz, S. A. Ozkan, I. Biryol, *Farmaco* **2000**, *55*(5), 376.
[20] S. Demircan, S. Kir, *Fabad J. Pharm. Sci.* **2005**, *30*(3), 116.
[21] G.-L. Chen, Y. -F. Li, F. -Y. Lee, *Chinese Pharm. J.* **2000**, *52*(2), 113.
[22] L. Valvo, R. Alimenti, S. Alimonti, S. Raimondi, F. Foglietta, F. Campana, *J. Pharm. Biomed. Anal.* **1997**, *15*(7), 989.
[23] P. M. Lacroix, S. J. Graham, E. G. Lovering, *J. Pharm. Biomed. Anal.* **1991**, *9*(10-8), 817.
[24] V. Das Gupta, *Drug Dev. Ind. Pharm.* **1985**, *11*(8), 1497.
[25] D. C. Tsilifonis, K. Wilk, R. Reisch R. E. Daly, *J. Liq. Chromatogr.* **1985**, *8*(3), 499.
[26] M.-E. Capella-Peiro, M. Font-Rubert, L. Alvarez-Rodriguez, J. Esteve-Romero, A. Durgbanshi, D. Bose, *J. Liq. Chromatogr. R. T.* **2007**, *30*(20), 2975.
[27] F. Faridbod, M. R. Ganjali, L. Safaraliee, S. Riahi, M. Hosseini, P. Norouzi, *Int. J. Electrochem. Sci.* **2009**, *4*(10), 1419.
[28] S. Demircan, S. Kir, S. A. Ozkan, *Anal. Lett.* **2007**, *40*(6), 1177.
[29] E. A. Kasim, M. A. Ghandour, M. T. El-Haty, M. M. Ahmed, *J. Pharm. Biomed. Anal.* **2002**, *30*(4), 921.
[30] J. A. Ortuno, C. Sanchez-Pedreno, A. Gil, *Anal. Chim. Acta* **2005**, *554*(1-2), 172.
[31] M. N. Abbas, H. S. Amer, *Curr. Pharm. Anal.* **2008**, *4*(2), 90.
[32] S. M. Sultan, A. M. Idris, K. E. Ibrahim, *J. Flow Injection Anal.* **2004**, *21*(1), 19.
[33] S. M. Sultan, A. M. Idris, K. E. Ibrahim, *J. Flow Injection Anal.* **2005**, *22*(2), 118.
[34] A. M. Idris, S. M. Sultan, K. E. Ibrahim, F. N. Assubaei, *J. Flow Injection Anal.* **2005**, *22*(2), 123.
[35] A. M. Idris, F. N. Assubaie, S. M. Sultan, *Microchem. J.* **2006**, *83*(1), 7.
[36] A. M. Idris, F. N. Assubaie, S. M. Sultan, *J. Autom. Methods Manage. Chem.* **2007**, article ID 32470, 7 pages.
[37] A. M. Idris, *J. Pharmacol. Toxicol. Methods* **2007**, *56*(3), 330.
[38] A. M. Idris, A. O. Alnajjar, *Talanta* **2008**, *77*(2), 522.
[39] F. N. Assubaie, *J. Food Drug. Anal.* **2009**, *17*(6), 408.
[40] A. M. Idris, *J. Anal. Chem.* **2010**, *65*(1), 36.
[41] A. M. Idris, *Chem. Central J.* **2011**, *5*, 9.
[42] ICH Harmonised tripartite guideline validation of analytical procedures: text and methodology Q2 (R1). Parent Guideline dated October 27, 1994 (complementary guideline on methodology dated November 6, **1996**, incorporated in November 2005). Available at: http://www.ich.org/LOB/media/MEDIA417.pdf [16 February 2011].
[43] International Union of Pure and Applied Chemistry. *Pure Appl. Chem.* **2002**, *74*(5), 835.