Spectrophotometric Determination of Methyldopa and Dopamine Hydrochloride in Pharmaceutical Preparations Using Flow Injection Analysis

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Abstract

Methyldopa and dopamine hydrochloride were determined spectrophotometrically in the pure form and in the pharmaceutical preparations using flow-injection analysis (FIA). The method is based on oxidative coupling reaction of drug with 2-furoic acid hydrazide in the presence sodium nitroprusside in sodium hydroxide medium to form a reddish-orange soluble product that has a maximum absorption at 487 nm. The various chemical and physical variables were optimized. The calibration graphs are linear from 1 to 100 μg mL^{-1} for methyldopa and dopamine hydrochloride. The detection limit (S/N = 3) was 0.769 and 0.560 μg mL^{-1} for methyldopa and dopamine hydrochloride, respectively. The method was successfully applied to the analysis of methyldopa and dopamine hydrochloride in tablets and injections preparations, respectively. The results obtained by applying the proposed FIA method were in good agreement with those obtained by British Pharmacopoeia method.

Keywords: Spectrophotometric; Flow-injection; Methyldopa; Dopamine hydrochloride; Pharmaceutical preparations.

الخلاصة

تم تقدير مثيل دوبا و هيدروكلوريد دوبامين طيفياً في الشكل النقي و في المستحضرات الصيدلانية باستخدام تحليل الحقن الجرياني. تعتمد الطريقة على تفاعل الازدواج التأكسدي للدواء مع 2-حامض الفيوريك هيدرازين و نتروبروسيد الصوديوم و في وسط هيدروكسيد الصوديوم. إذ يتكون ناتج برتقالي محمر ذائب في الماء يمتلك أقصى امتصاص عند طول موجي 487 نانومتراً. تم تثبيت المتغيرات الكيميائية والفيزيائية للحصول على أفضل حساسية و تطابقية للنتائج. تم الحصول على منحنيات معايرة خطية من 1 - 100 مكغم مل$^{-1}$ لكلا الدوائين مع قيم حد كشف 0.769 و0.560 مايكروغرام مل$^{-1}$ لمثيل دوبا و لهيدروكلوريد دوبامين على التوالي. طبقت الطريقة بنجاح في تقدير كلا الدوائين في المستحضرات الصيدلانية و كانت النتائج المستحصلة متوافقة مع النتائج المستحصلة من الطريقة القياسية المعتمدة من قبل دستور الأدوية البريطاني و عند مستوى ثقة 95 %.

Introduction

Methyldopa (α-methyl-3,4-dihydroxyphenyl alanine) is a centrally acting antihypertensive agent and dopamine (3,4-dihydroxyphenylethylamine) is a central neurotransmitter particularly important in the regulation of movement and possesses important intrinsic pharmacological properties. It is used for the correction of hemodynamic disorders associated with shock episodes[1].

Various methods for the determination of methyldopa and dopamine hydrochloride in pharmaceutical preparations have been reported in the literature including potentiometry[2, 3], titrimetry[4], high-performance liquid chromatography[5, 6], ^{1}H nuclear magnetic resonance spectroscopy[7], chemiluminescence[8], fluorometry[9-11], voltammetry[12] and spectrophotometry[13-19]. A number of flow-injection (FI) methods have also been reported for the determination of these drugs, such as FI-spectrophotometry[20-26], FI-chemiluminescence[27-30] and FI-amperometry[31, 32]. However, the control of such reactions and / or manifolds is still complicated.

Most of spectrophotometric methods for determination methyldopa and dopamine hydrochloride are time consuming and require heating. In most of these methods, absorbance measurements for both samples and standards must be done either at a constant, fixed time after addition of the colorimetric reagent or waiting for the reaction to proceed to completion in order to attain the required reproducibility.

In this work, we have demonstrated the possibility of using flow injection analysis (FIA) to overcome these difficulties. In FIA, reaction completion is not necessary because measurements for all samples and standards are subjected to the same timing sequence in a precise, automatic manner. FIA technique has found recently wide applications mainly due to reduction of the analysis time and reagent consumption compared with conventional manual procedures.

In this paper FI method using spectrophotometric detection at 487 nm are described for the determination of methyldopa and dopamine hydrochloride. The batch method[33] was adopted as a basis to developed FIA method. The method is based on oxidative coupling reaction of drug with 2-furoic acid hydrazide (2-FAH) and sodium nitroprusside (SNP) in sodium hydroxide medium to form a reddish-orange soluble product. The FI method has been successfully applied to the determination of methyldopa and dopamine hydrochloride in pharmaceutical preparations.

Experimental

Apparatus

A Shimadzu UV-VIS 260 (Tokyo, Japan) digital double-beam recording spectrophoto-meter was used for all spectral and absorbance measurements with matched 1-cm quartz cells.

The FI system comprised a peristaltic pump (Ismatec, Labortechnik-Analytic, CH-8152, Glatbrugg-Zurich, Switzerland, six channels) with polyvinyl chloride flow tubes of 0.8 mm i.d., an injection valve (Rheodyne, Altex 210, Supelco-USA), a 50 µL flow cells and a Shimadzu UV-VIS 260 spectrophotometer (Tokyo, Japan) as the detector. Flexible Teflon tubes of 0.5 mm i.d. were used for reaction coils and to transport the reagents solutions. T-link was also used to mix two streams of reagents.

Reagents

All chemicals were of analytical reagent grade.

1- Drug stock standard solution 500 µg mL^{-1} was prepared by dissolving 0.0500 g of pure methyldopa (SDI) or dopamine hydrochloride (Fluka) in distilled water and diluting to the mark with the same solvent in 100 mL volumetric flask. Working standard solutions were prepared by suitable dilution of the stock standard solution.
2- Sodium nitroprusside (SNP) solution 7×10^{-3} M was prepared by dissolving 0.5213 g of SNP (Riedel-dehaen) in distilled water and diluting to the mark with the same solvent in 250 mL volumetric flask.
3- 2-Furoic acid hydrazide (2-FAH) solution 5×10^{-3} M was prepared by dissolving 0.1261 g of 2-furoic acid hydrazide (Aldrich Chemical Co. Ltd.) in distilled water and diluting to the mark with the same solvent in 200 mL volumetric flask.
4- Sodium hydroxide solution 0.05 M was prepared by dissolving 0.5000 g of sodium hydroxide (BHD) in distilled water and diluting to the mark with the same solvent in 250 mL volumetric flask.

More dilute solutions were prepared by appropriate dilutions using distilled water. **Pharmaceutical preparations**

Pharmaceutical preparations were obtained from commercial sources.

1- ***Aldomethyl tablets (Asia - Syria):*** 250 mg methyldopa for each tablet.
2- ***Dopamine hydrochloride injections (Biologici Italy Lab., Novate, Milano - Italy):*** 200 mg dopamine hydrochloride for each injection (5 mL).

Recommended procedure for calibration FI-procedure

The FI system is shown in Fig. (1). 150 µL aliquots of drug solutions prepared at different concentrations (1 – 100 µg mL^{-1}) were injected into carrier stream which produced from mixing of two channels. The first channel was used to transport SNP solution of 7×10^{-3} M and second channel was used to transport mixture solution from 5×10^{-3} M of 2-FAH solution and 0.05 M of sodium hydroxide. The total flow rate of the two channels was 1.5 mL min^{-1}. The reaction was carried out by passing the solution through a reaction coil (75 cm) and the absorbance of the resulting reddish-orange color product was measured at 487 nm. Calibration graphs of methyldopa and dopamine hydrochloride were prepared by plotting the absorbances of the peak maximum versus drug concentrations.

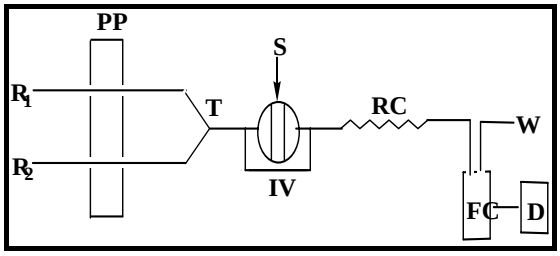

Fig. (1): FI manifold for determination of methyldopa and dopamine hydrochloride (R_1 = SNP, R_2 = 2-FAH + NaOH, S = Sample injection, PP = Peristaltic pump, IV = Injection valve, T = T-link, RC = Reaction coil, FC = Flow cell, D = Detector and W = Waste)

Procedure for the assay of pharmaceutical preparations

1- Tablets solution (500 µg mL^{-1})

The average tablet weight was calculated from the contents of 10 tablets that have been finely powdered and weighed. A portion of this powder, equivalent to 50 mg of methyldopa, was accurately weighed. The sample was dissolved and diluted with distilled water in a 100 mL volumetric flask. The later solution was filtered twice.

Injections solution (500 µg mL^{-1})

The contents of three injections were mixed. An aliquot corresponding to 50 mg of dopamine hydrochloride (2.5 mL) was diluted to 200 mL with distilled water in a volumetric flask.

Further appropriate solutions of pharmaceutical preparations were made by using distilled water. Two different concentrations of each solution of pharmaceutical preparation were analyzed in five replicate by recommended FI spectrophotometric procedure.

Results and Discussion

Preliminary studies

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configurations and the approximate ranges of the tested parameters. The design of the manifold selected is shown in Fig. (1) using total flow rate of 1.5 mL min^{-1} for two-channels. This design of the manifold gave the maximum absorbance. Therefore, a two-channel FI assembly was adopted, in which the sample (100 µL) was injected into the carrier stream, which was formed from mixing two carrier streams (R_1 and R_2). The reaction was carried out by passing the solution through a reaction coil (75 cm) and the absorbance of the resulting reddish-orange color product was measured at 487 nm. The presence of the drug caused an increase in the absorbance, which was proportional to its concentration.

Optimization of the experimental conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of methyldopa by FI method.

The effect of the concentration of SNP was studied in the range $1 \times 10^{-3} - 2 \times 10^{-2}$ M with fixed methyldopa concentration of 20 µg mL^{-1}. As can be observed from Fig. (2) the absorbance was increased as the concentration of SNP was increased up to 7×10^{-3} M, thus 7×10^{-3} M SNP was found to be the most suitable concentration for a maximum absorbance and was chosen for further use.

The effect of the concentration of sodium hydroxide was studied in the range 0.01 – 0.2 M. As can be observed from Fig. (2) the absorbance was increased as the concentration of sodium hydroxide was increased up to 0.05 M, thus 0.05 M sodium hydroxide was found to be the most suitable concentration for a maximum absorbance and was chosen for further use.

It was found that the reaction between methyldopa and 2-FAH and SNP in sodium hydroxide medium depends on the 2-FAH concentration. Therefore, the effect of different concentrations of 2-FAH ($1 \times 10^{-3} - 2 \times 10^{-2}$ M) was studied [Fig. (2)]. The result obtained indicated, that the absorbance increased with the increasing concentration of 2-FAH up to 5×10^{-3} M, thus a concentration of 5×10^{-3} M gave the maximum absorbance and was chosen for further use.

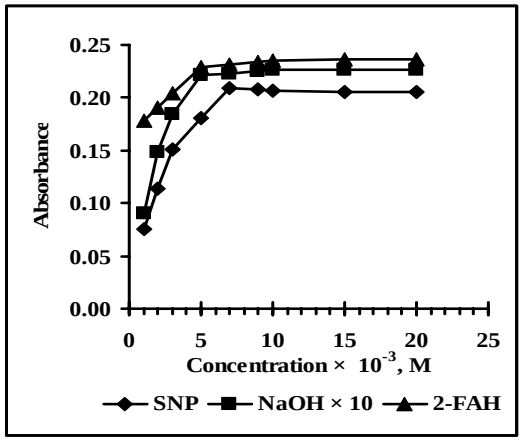

Fig. (2): Chemical conditions of FI procedure for determination of methyldopa

The use of FI as an alternative to existing methods for methyldopa determination is dependent on optimization of the system to achieve maximum absorbance. As a consequence, several experiments were conducted in order to establish the best experimental conditions for operating the FI manifold.

Fig. (3) shows the effects of flow rate, reactor length and sample injection volume on the absorbance. The effect of flow rate on the absorbance was studied over the range 0.5 – 3.5 mL min^{-1}. Fig. (3) shows that, with increasing flow rate, maximum sensitivity was obtained at 1.5 mL min^{-1}, which was selected, as a compromise between reproducibility and sampling rate. Above this value, the absorbance decreased slightly owing to dispersion effects.

The effect of reactor length was studied in the range 25 – 200 cm in the same experimental conditions selected above. As can be seen from Fig. (3), maximum absorbance value was obtained at 75 cm and was selected for further use.

The volume of sample injected was varied in the range 50 – 250 µL by changing the length of the sample loop in the injection valve, while the other variables remained constant. The absorbance increased with increasing volume of sample injected [Fig. (3)]. Best sensitivity was obtained by using 150 µL as a volume of sample injected, which was selected.

The flow system selected provided a sampling rate of 40 samples h^{-1}.

Table (1): Data for the calibration graphs for paracetamol using the proposed methods

Parameter	Value	
	Methyldopa	Dopamine hydrochloride
Linearity range (µg mL^{-1})	1 – 100	1 – 100
r	0.9996	0.9997
r^2	0.9993	0.9995
a (mL µg $^{-1}$)	0.0131	0.0190
b	0.0042	0.0076
$S_{y/x}$	1.2954×10^{-2}	1.7450×10^{-2}
S_a	1.2397×10^{-4}	1.5520×10^{-4}
S_b	5.9491×10^{-3}	8.0539×10^{-3}
E%	0.102*	0.857**
RSD%***	0.651	0.569

*** For 60 µg mL^{-1} of methyldopa.**
**** For 70 µg mL^{-1} of dopamine hydrochloride.**
***** Average of five determination.**

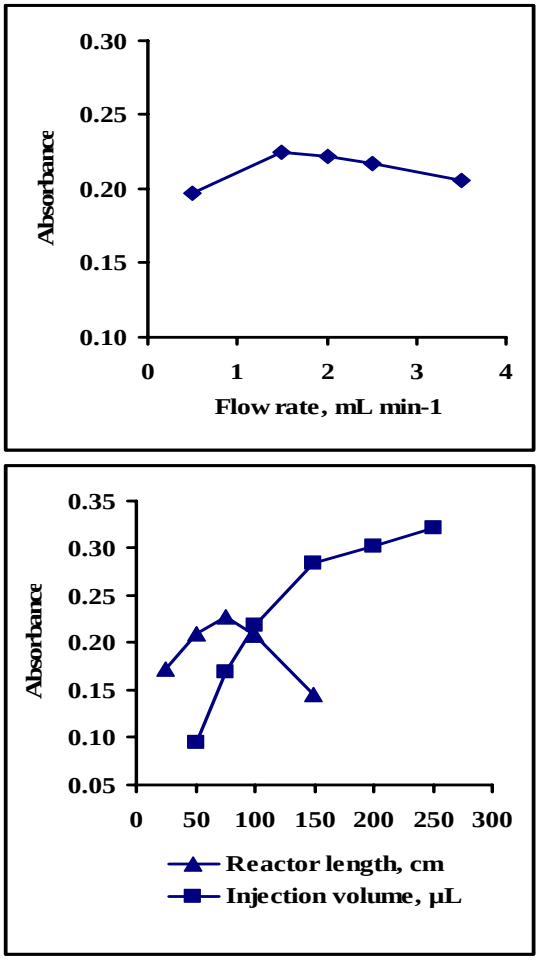

Fig. (3): Physical conditions of FI procedure for determination of methyldopa

Analytical characteristics of FI spectrophotometric method

For FI method, the calibration graphs for methyldopa and dopamine hydrochloride were obtained by the procedure described previously in which a series of standard solutions were analyzed in triplicates to test the linearity. The slope (a), the intercept (b), the correlation coefficient (r) and the correlation of determination (r^2) were evaluated by a least-squares regression analysis and are included in Table (1).

Statistical evaluation[34] of the regression line gave the values of standard deviations for residuals ($S_{y/x}$), slope (S_a) and intercept (S_b) at 95% confidence are shown in the same Table. These small figures point out to the high precision of the proposed method.

Table (2): Pharmaceutical applications for methyldopa and dopamine hydrochloride using the proposed method

Pharmac e-utical Preparati on	Concn. of drug (µg mL^{-1})*		E,%	Rec.,%	RSD, %
	Present	Found			
Aldometh yl tablets	10.000	9.931	– 0.689	99.310	1.179
	20.000	20.027	+ 0.139	100.139	0.666
	30.000	30.074	+ 0.247	100.148	0.301
Dopamine hydrochlo ride injections	5.000	4.916	– 1.680	98.320	1.642
	20.000	19.968	– 0.160	99.840	0.517
	30.000	30.389	+ 1.297	101.297	0.436

*** Average of five determination.**

Accuracy and precision of the batch and FI spectrophotometric methods

The accuracy and precision of the proposed method was tested by analyzing five replicate samples of methyldopa and dopamine hydrochloride. The values of the percentage errors (E%) and percentage relative standard deviation (RSD%) are summarized in Table (1). These values indicate the high accuracy and precision of the proposed method.

Pharmaceutical applications

In order to demonstrate the applicability of the proposed method for the determination of methyldopa and dopamine hydrochloride, the method was successfully applied to the analysis of methyldopa in tablets and dopamine hydrochloride in injections. The results are summarized in Table (2). When pharmaceutical preparations of methyldopa and dopamine hydrochloride were analyzed by the proposed method, interference from the sample matrix caused no problem. For all the formulations examined, the assay results of proposed method were in good agreements with the declared contents. In two drugs, quantitative recoveries between 98.320 and 101.297% were obtained [Table (2)].

The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method[35] [Table (3)] by applying the F-test and the t-test at 95% confidence level[34]. The calculated values for F and t for methyldopa and dopamine hydrochloride (5.372, 0.785 and 13.136, 0.903 respectively), did not exceed the critical values of $F_{1,\ 1}$ = 161.4 and t = 4.303 ($n_1 + n_2 - 2 = 2$). These confirming that there are no significant differences between the proposed method with BP method with respect to precision and accuracy in the determination of methyldopa and dopamine hydrochloride in pharmaceutical preparations.

Conclusions

The FI spectrophotometric method proposed for the determination of methyldopa and dopamine hydrochloride in pure and pharmaceutical forms has the advantages of simplicity, speed, accuracy and the use of inexpensive equipment.

The speed of analysis and the precision render this method also suitable for the quality control of formulations containing these drugs replacing tedious, expensive and slow official and chromatographic method. There is no significant difference between the proposed method with respect to precision and accuracy.

Table (3): Comparison of the proposed method with BP method for determination of pharmaceutical preparations

Pharmaceutical Preparation	Rec., %*	
	FI method	BP method
Pure methyldopa	100.269	100.000
Aldomethyl tablets	99.866	100.934
Pure dopamine hydrochloride	100.288	100.000
Dopamine hydrochloride injections	99.819	101.700

***Average of five determinations.**

References

1. Gilman, A. G., ***"The Pharmacological Basis of Therapeutics"***, 1996, 9th ed., McGraw-Hill, New York, 211.
2. Athanasiou-Malaki, E. M. and Koupparis, M. A., ***Anal. Chim. Acta***, 1984, **161**, 349.
3. Othman, A. M., Rizka, N. M. H. and El-Shahawi, M. S., ***Anal. Sci.***, 2004, **20**, 651.
4. Salem, F. B., ***Talanta***, 1987, **34**, 810.
5. Dolez' alova´, M. and Tkaczykova, M., ***J. Pharm. Biomed. Anal.***, 1999, **19**, 555.
6. Zecˇevic´, M., Zivanovic, Lj., Agatonovic-Kustrin, S. and Minic, D., ***J. Pharm. Biomed. Anal.***, 2001, **24**, 1019.
7. Talebpour, Z., Haghgoo, S. and Shamsipur, M., ***Anal. Chim. Acta***, 2004, **506**, 97.
8. Arakawa, H., Kanemitsu, M. and Maeda, M., ***Anal. Sci.***, 1999, **15**, 1269.
9. Zhang, D., Fu, M., Ma, W. and Chen, D., ***Anal. Sci.***, 2001, **17**, 1331.
10. Wang, H. Y., Hui, Q. S., Xu, L. X., Jiang, J. G., Sun, Y., ***Anal. Chim. Acta***, 2003, **497**, 93.
11. Wang, H. Y., Feng, X. G., Zhang, M. and Zhao, H., ***Anal. Sci.***, 2007, **23**, 1297.
12. Chernyshov, D. V., Shvedene, N. V., Antipova, E. R., Pletnev, I. V., ***Anal. Chim. Acta***, 2008, **621**, 178.
13. Davidson, A. G., ***J. Pharm. Biomed. Anal.***, 1984, **2**, 45.
14. Sastry, C. S. P., Das, V. G. and Rao, K. E., ***Analyst***, 1985, **110**, 395.
15. Helaleh, M. I. H., Rahman, N. and Abu-Nameh, E. S. M., ***Anal. Sci.***, 1997, **13**, 1007.
16. Nagaraja, P., Srinivasamurthy, K. C., Rangappa, K. S. and Growda, N. M., ***Talanta***, 1998, **46**, 39.
17. Vieira, I. C. and Fatibello-Filho, O., ***Talanta***, 1998, **46**, 559.
18. Li, Q., Li, J. and Yang, Z., ***Anal. Chim. Acta***, 2007, **583**, 147.
19. Chamsaz, M., Safavi, A. and Fadaee, J., ***Anal. Chim. Acta***, 2007, **603**, 140.
20. Berzas, J. J., Lemus, J. M. and Buitrage, P., ***Fresenius J. Anal. Chem.***, 1995, **353**, 221.

21. Tubino, M., Junior, A. R. and Vila, M. M. D. C., ***J. Flow injection Anal.***, 2004, **21**, 132.
22. M. Q. Al-Abachi and M. A. Al-Da'amy, ***National J. Chem.***, 2004, **13**, 47.
23. M. Q. Al-Abachi and M. A. Al-Da'amy, ***National J. Chem.***, 2004, **15**, 339.
24. M. Q. Al-Abachi and M. A. Al-Da'amy, ***National J. Chem.***, 2004, **16**, 485.
25. Ribeiro, P. R. S., Neto, J. A. G., Pezza, L. and Pezza, H. R., ***Talanta***, 2005, **67**, 240.
26. Abdulrahman, L. K., Al-Abachi, A. M. and Al-Qaissy, M. H., ***Anal. Chim. Acta***, 2005, **538**, 331.
27. Wang, S., Du, L., Wang, L. and Zhuang, H., ***Anal. Sci.***, 2004, **20**, 315.
28. Yu, C., Tang, Y., Han, X. and Zheng, X., ***Anal. Sci.***, 2006, **22**, 25.
29. Liu, Y. and Song, Z., ***Can. J. Anal. Sci. Spectrosc.***, 2006, **51**, 59.
30. Nalewajko, E., Wiszowata, A. and Kojlo, A., ***J. Pharm. Biomed. Anal.***, 2007, **43**, 1673.
31. Bezerra, V. S., Filho, J. L. L., Montenegro, M. C. B. S. M., Arau´jo, A. N., Silva, V. L., ***J. Pharm. Biomed. Anal.***, 2003, **33**, 1025.
32. Yeh, W. L., Kuo, Y. R. and Cheng, S. H., **Electrochem. Communi.**, 2008, **10**, 66.
33. Al-Esawati, F. M. J., ***"Master Thesis"***, University of Mosul, 2002.
34. Miller, J. N. and Miller, J. C., ***"Statistics and Chemometrics for Analytical Chemistry"***, 2000, 4th ed., Pearson Education Limited, London.
35. ***"British Pharmacopoeia on CD-ROM"***, 2001, 3rd ed., The Stationery Office Ltd., London.