Chemometrics –Assisted Simultaneous Determination of Atenolol and Furosemide in Synthetic Binary Mixtures and Combined Tablet Preparations

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Abstract: In this work a numerical method, based on the use of spectrophotometric data coupled to PLS multivariate calibration, is reported for the simultaneous determination of furosemide (FUR) and atenolol (ATE) in synthetic samples and combined commercial tablets. The correlation coefficients (R^2) and recovery range for ATE and FUR in synthetic mixtures were 0.9938, 0.9949 and 94.72-103.72%, 95.60-104.34% respectively. The results of optimized method in combined tablet preparations compared with British Pharmacopeia 2007 standard methods that have a good agreement. The proposed method are simple, fast, inexpensive and do not need to any separation or preparation methods.

Keywords: Atenolol, Furasemide, Spectrophotometric, Multivariate calibration.

Introduction

Atenolol (RS)-2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide (ATE) is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers (sometimes written β -blockers), a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, atenolol was developed as a replacement for propranolol in the treatment of hypertension. Furasemide 4-chloro-2-(furan-2-ylmethylamino)- 5-sulfamoylbenzoic acid (FUR) is a loop diuretic used in the treatment of congestive heart failure and edema. Along with some other diuretics, furosemide is also included on the World Anti-Doping Agency's banned drug list due to its alleged use as a masking agent for other drugs¹.

The determination of these drugs is a frequent analytical problem in quality control of the pharmaceutical industries. The two drugs studied in this work show a strong overlap between their absorption spectra. Hence, their simultaneous determination using conventional spectrophotometric techniques would be hard. Mainly, HPLC is used to resolve a complex mixture of these drugs².

Partial Least Squares regression (PLS) was originally developed by Wold³ and the use of PLS method for chemical analysis was pioneered by Wold *et al*⁴⁻⁵. A detailed description on the mathematical principles of the PLS algorithms have been reported by Martens *et al*⁶.

In recent years multivariate calibration methods have been used to resolve mixtures of two or more compounds with similar spectra characteristics. In most cases, multivariate methods for evaluation of spectroscopic data have more advantages as simplicity and cheapness. These methods have been applied for determination of drugs⁷⁻¹¹, because HPLC methods and conventional spectroscopic methods were slow, expensive and complex.

The aim of this paper is to investigate the ability of PLS method for quantifying binary mixtures of atenolol and furosemide without prior separation and to apply the optimized method in pharmaceutical preparations.

Experimental

Commercial samples were bought from pharmacies. Analytical grade ATE and FUR were obtained from Jalinous and Mehr-Daru pharmaceutical companies (Tehran, Iran). All other chemical and solvents were of analytical reagent grade.

Apparatus and software

A V-530 Jasco scanning spectrophotometer connected to PC fitted data software was used for all the measurements and treatment of data. The Matlab 7.1 and Unscrambler 9.1 softwares were used for the statistical treatment of the data and application of various multivariate methods.

Procedure

The 500 mg/L ATE and FUR stock solutions were prepared by dissolving accurately weighed amounts of finely powdered pure ATE and FUR in deionized water and 1.6×10^{-3} M NaOH respectively. The calibration and test mixtures prepared by mixing these two solutions with different ratios.

For 50 mg ATE and 40 mg FUR tablets, 20 of each tablets mixed and weighed. After grinding, mixing and homogenizing of an accurately weighed 20 tablets from each pharmaceutical products, 1/20 of sample was used for analysis. Each weighed sample was mixed with 40 mL deionized water and the mixture was subjected to ultrasonication for 15 minutes. After cooling and further dilution to 100 mL with deionized water, a 10 mL portion of the sample was centrifuged at 3000 rev/min. 0.25 mL portion of the supernatant was then diluted to 25 mL with deionized water.

For 100 mg ATE and 40 mg FUR tablets, 20 of each tablets mixed and weighed. After grinding, mixing and homogenizing of an accurately weighed 20 tablets from each pharmaceutical products, 1/40 of sample was used for analysis. Each weighed sample was mixed with 40 mL deionized water and the mixture was subjected to ultrasonication for 15 minutes. After cooling and further dilution to 100 mL with deionized water, a 10 mL portion of the sample was centrifuged at 3000 rev/min. 0.20 mL portion of the supernatant was then diluted to 25 mL with deionized water. The absorption spectra between 200 and 400 nm against blank were recorded for all solutions. Commercial tablets were also analyzed, using British Pharmacopoeia 2007 (BP2007) method¹².

The stability of ATE, FUR and commercial sample solutions were checked for 4 h and the UV–Vis absorption spectra of all sample solutions were found to be stable for this period of time. It is also to be noted that the simultaneous determination of the aforementioned drugs with the proposed method can be carried out in less than 1 h.

Results and Discussion

UV-Vis spectra of ATE and FUR

In figure 1, the absorption spectra of ATE and FUR solutions in methanol recorded between 200 and 400 nm are shown. The two drugs studied show a strong overlap in their absorption spectra and the conventional spectrophotometric methods can not be applied for resolving this mixture.

Figure 1. The UV-Vis absorption spectra ATE, FUR and mixture of tablets solutions.

Experimental design of sample sets

Calibration and test sets for two component systems were designed according to factorial principle. Solutions containing drug concentrations in the range 0.0–10.0 mg/L for ATE and FUR were produced by dilution of the stock solutions. A five-level factorial design was used to produce a full set of 25 samples. A three-level set was derived to produce a calibration set of nine samples, with the remaining 16 samples used for an independent test set¹³. The compositions of the used calibration and test sets are summarized in Table 1 and Table 2, respectively.

Table 1. Calibration set composition.

Standard	ATE, mg/L	FUR, mg/L
C ₁	0	0
C ₂	0	5
C ₃	0	10
C ₄	5	0
C ₅	5	5
C ₆	5	10
C ₇	10	0
C ₈	10	5
C ₉	10	10

Table 2. Test set composition.

Sample	ATE, mg/L	FUR, mg/L
T ₁	0	2.5
T ₂	0	7.5
T ₃	2.5	0
T ₄	2.5	2.5
T ₅	2.5	5
T ₆	2.5	7.5
T ₇	2.5	10
T ₈	5	2.5
T ₉	5	7.5
T ₁₀	7.5	0
T ₁₁	7.5	2.5
T ₁₂	7.5	5
T ₁₃	7.5	7.5
T ₁₄	7.5	10
T ₁₅	10	2.5
T ₁₆	10	7.5

Selection of the optimum number of factors and the spectral region

To select the correct number of factors in the PLS algorithm, a cross-validation method, leaving out one sample at a time, was employed. The predicted concentrations $\bar{X}_{ij}(k)$ of the compounds in each I sample, obtained with k factors, were compared with the already known concentrations X_{ij} and the root mean squared error of cross validation (RMSECV) was calculated for each of the k factor levels as follows:

$$RMSWC(V) = \sqrt{\frac{\sum_{i=1}^n \sum_{j=1}^m (\bar{X}_{ij}(k) - X_{ij})^2}{1}}$$

The optional value for k is the level that yields the smallest RMSECV(k) value. To check the selected factor number, the external validation method was also used by simply computing the root mean squared error of predication (RMSEP) for L objects in test set for each of the k factor levels as follows:

$$RMSEP(k) = \sqrt{\frac{\sum_{i=1}^n \sum_{j=1}^m (\bar{Z}_{ij}(k) - Z_{ij})^2}{L}}$$

where Z_{ij} is the known concentration in the test set and $\bar{Z}_{ij}(k)$ is predicted for concentrations with k factors⁷. This showed that the external test set validation and the internal cross-validation indicated about the same number of factors.

To select the spectral region, all of the above steps used repeatedly and the spectral region that lead to the lowest values of RMSEP was selected⁷. The optimal number of factors, RMSEP and RMSECV values and optimum spectral regions obtained by PLS-1 and PLS-2 algorithms are summarized in Table 3.

The proposed PLS-1 and PLS-2 calibration models were evaluated by prediction of drug concentrations in their own designed calibration set. Recoveries values were between 96.26 and 105.28% for ATE and between 95.52 and 103.57% for FUR.

Table 3. Optimal number of factors, RMSEP and RMSECV values.

		Factor No.	RMSEP	Factor No.	RMSECV
PLS-1	ATE	2	0.2348	2	0.2406
212-243 nm	FUR	2	0.2712	2	0.2971
PLS-2	ATE	2	0.2299	2	0.2389
212-243 nm	FUR	2	0.2355	2	0.2230

Statistical parameters for the optimized models

Using the internal validation in their own designed calibration set, the following statistical parameters have been obtained:

- The values of root mean squared error of calibration (RMSEC), which is an indication of the average error in the analysis for each component.
- The square of correlation coefficients (R^2), which is an indication of the quality of the straight line that fits the data.

In Table 4, the results obtained for these parameters by PLS-1 and PLS-2 are shown. We can see that the R^2 values are in all cases very near to 1 which is an indication of similarity between predicted and known values. On the other hand, in general terms, the statistical parameters obtained by PLS-1 and PLS-2 are good.

Table 4. Statistical parameters of the model optimized

	PLS-1		PLS-2	
	RMSEC	R^2	RMSEC	R^2
ATE	0.2909	0.9921	0.2948	0.9919
FUR	0.2868	0.9970	0.1051	0.9993

External validation of PLS-1 and PLS-2 calibration models

16 Synthetic mixtures in test set were predicted by applying PLS-1 and PLS-2 methods. The square of correlation coefficients (R^2) and recovery range for these synthetic solutions are summarized in Table 5. Satisfactory values are obtained in most of mixtures analyzed by the methods. Limits of detection (LOD's) were calculated as three times of standard error of estimation (SEE) values⁷. LOD's of 0.933 mg/L for ATE, 0.919 mg/L for FUR, 0.945 mg/L for ATE, and 0.929 mg/L for FUR were obtained in PLS-1 and PLS-2 models, respectively.

Table 5. Recovery range and R^2 for synthetic mixtures in test set.

	ATE		FUR	
	%Rec. range	R^2	%Rec. range	R^2
PLS-1	94.36-103.40	0.9937	95.53-103.76	0.9921
PLS-2	94.72-103.72	0.9938	95.60-104.34	0.9949

Analysis of commercial samples

Commercial mixture products were analyzed using two methods: the proposed spectrophotometric method and BP2007 method¹². Results are summarized in Table 6. As can be seen, satisfactory results were obtained in all cases by the proposed methods.

Table 6. Analysis of commercial tablets mixtures.

	Commercial tablet mixtures		Commercial tablet mixtures	
	ATE±%RSD	FUR±%RSD	ATE±%RSD	FUR±%RSD
Declared contents*	50	40	100	40
PLS-1 Results	50.11±1.01	36.15±1.21	102.58±1.01	36.66±1.40
PLS-2 Results	50.17±1.10	36.55±1.00	102.47±1.20	36.38±1.10
BP2007 Results	49.00±1.30	36.00±1.40	98.00±1.30	36.00±1.40

*Results presented as mg per tablet.

Statistical parameters obtained by replicate analysis, for the commercial tablets with proposed PLS-1, PLS-2 and BP2007 standard methods are also given in Table 6. These results show that the precision of the proposed methods and BP2007 method are same.

Conclusion

A comparative study of the use of PLS-1 and PLS-2 for the resolution and simultaneous determination of ATE and FUR in a binary mixture has been accomplished, showing that this method provide a clear example of the high resolving power of these techniques. In several terms, similar results were obtained for these two drugs in both synthetic and commercial mixture applications by PLS-1 and PLS-2.

The results obtained confirm the suitability of the proposed method for accurate and precise analysis of Atenolol and Furosemide in pharmaceutical preparations. These methods were applied directly to the commercial mixture preparations without previous treatment. Also no expensive (dissolution and injection) laboratory technique is needed. In addition the proposed methods are suitable for application without interference of the excipients.

References

1. www.fda.gov
2. Bansal T, Singh M, Mishra G, Talegaonkar S, Khar R K, Jaggi M and Mukherjee R, *J Chromatogr B*, 2007, **859**(2), 261-266.
3. Wold H, in *Research Papers in Statistics*, (David F, Eds), Wiley, New York, 1966.
4. Wold H, in *Systems under Indirect Observations*, (H. Joreskog and H. Wold, Eds), North-Holland, Amesterdam, 1982.
5. Wold S, Martens H and Wold H, in *The Multivariate Calibration Problem in Chemistry solved by PLS, Matrix Pencils (Lecture Notes in Mathematics)*, (A. Ruhe and Kagstrom B, Eds), Springer, Heidelberg, 1992.
6. Martens H and Naes T, *Multivariate Calibration*, Wiley, Chichester, 1992.
7. Kargosha K and Sarrafi A H M, *J Pharm Biomed Anal.*, 2001, **26**, 273-279.
8. Aksu O, Bozdogan A and Kunt G, *Anal Lett.*, 2006, **39**,751.
9. Hadad G M, El-Gindy A and Mahmoud W M M, *Spectrochim Acta Part A*, 2008, **70**, 655-663.
10. Khoshayand M R, Abdollahi H, Shariatpanahi M, Saadatfard A and Mohammadi A, *Spectrochim Acta Part A.*, 2008,**70**,491-499,
11. Sarrafi A H M, Khodakarami Z and Karkeabadi M, *E Journal of Chemistry*, 2009, **6**(S1), S111-S116.
12. British Pharmacopeia 2007, CD-ROM.
13. Bayne C K and Rubin I B, *Experimental Design*, VCH, New York, 1986.