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Simultaneous determination of fosinopril and hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatographic methods

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Abstract

Fourth derivative UV-spectrophotometric and high-performance liquid chromatographic (HPLC) methods for simultaneous determination of fosinopril and hydrochlorothiazide in tablets have been developed. Standard solutions were measured at zero crossing wavelengths of 217.7 and 227.9 nm for fosinopril and hydrochlorothiazide, respectively, by fourth derivative spectrophotometric method. Calibration curves were constructed by plotting $d^4A/d\lambda^4$ values at selected wavelengths against concentrations. HPLC analyses were carried out on C-18 column with gradient elution by using 10 mM H_3PO_4 and CH_3CN as mobile phase. Benazepril was used as internal standard and the substances were detected at 215 nm. Commercially available tablets containing 20 mg fosinopril and 12.5 mg hydrochlorothiazide were analysed by fourth derivative spectrophotometric and HPLC methods. The results were compared statistically at 95% confidence level with each other. There was no significant difference between the mean percentage recoveries and precision of the two methods.

Keywords: Fosinopril; Hydrochlorothiazide; UV-derivative spectrophotometry; High-performance liquid chromatography

1. Introduction

Fosinopril (Fos), an angiotensin-converting enzyme inhibitor used to treat hypertension, and hydrochlorothiazide (Hct), a diuretic agent, are together used in some commercial preparations. Only a multi-wavelength UV-spectrophotometric method has been developed [1] for simultaneous determination of these drugs and there is no official method in pharmacopoeias for this purpose. On the other hand, although various methods have been reported for the assay of hydrochlorothiazide which has been used since a long time in therapy, a liquid chromatographic method [2] has been presented for Fos more recently. Some quantitative determination methods of Hct are spectrophotometric [3–6], fluorodansitometric [7], gas [8,9] and liquid [10–12] chromatographic, electrophoretic [13] and polarographic [14,15] methods.

In this study, a fourth derivative spectrophotometric and HPLC methods were developed for simultaneous determination of Fos and Hct in tablets. These methods were especially chosen since these are used for the determination of drugs in pharmaceutical preparations reported in pharmacopoeias. The results obtained with these methods were statistically compared.

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2. Experimental

2.1. Materials

Pharmaceutical grade Fos sodium and Hct (Bristol-Myers Squibb Co.) and benazepril hydrochloride (Ben), internal standard (Ciba Geigy) were kindly supplied by the manufacturers. Analytical grade H_3PO_4 and HPLC grade MeOH and CH_3CN were purchased from Merck (Darmstadt). Water was doubly distilled.

2.2. Apparatus

A Shimadzu UV-160A UV–VIS spectrophotometer with 1 cm quartz cells was used under the following operating conditions: scan speed 1500 nm min^{-1}, scan range 200–300 nm, slit width 2 nm and derivation interval ($\Delta\lambda$) 2.4 nm. Derivative spectra were automatically obtained by Shimadzu UV-160A system software.

HPLC analyses were performed on a Shimadzu LC 10 (Tokyo) system. This consisted of a model LC 10 AT solvent delivery system, a Rheodyne injector with a loop of 20 μl. SPD-10A spectrophotometric detector was set at 215 nm. The data were collected and analysed via the automation system software (Shimadzu).

2.3. Chromatographic conditions

Separation was achieved at room temperature, using a Shim-pack C-18 column (250 mm × 4.6 mm i.d., 10 μm) (Shimadzu). The mixture of aqueous 10 mM *o*-phosphoric acid (solvent A) and acetonitrile (solvent B) was used for gradient elution. Sixty percent of A was passed through the column for 4 min with a flow rate 1 ml min^{-1}, then concentration of A was gradually changed to 20% A over 6 min. At the fifth minute of the run the flow rate was increased to 2 ml min^{-1}. The waiting time between the runs was 10 min to obtain good stabilized conditions.

2.4. Solutions

Fosinopril and hydrochlorothiazide stock solutions (0.5 mg ml^{-1} in MeOH) were freshly prepared. Standard solutions were obtained by diluting the stock solutions for the preparation of calibration curves in the concentration range of 5.0–45.0 μg ml^{-1} (Fos) and 0.5–9.0 μg ml^{-1} (Hct) for derivative spectrophotometric method. Meanwhile the final concentration ranges were 5.0–50.0 μg ml^{-1} (Fos) and 2.5–25.0 μg ml^{-1} (Hct) for HPLC method. These solutions also contained benazepril as internal standard at 15 μg ml^{-1}.

2.5. Assay procedure

Ten tablets were separately weighed and powdered. About 60 ml of MeOH was added to accurately weighed amount of the tablet powder equivalent to approximately 20 mg of Fos and 12.5 mg of Hct in a 100 ml calibrated flask. The mixture was shaken for 30 min on a shaker, diluted to volume with MeOH, and then filtered.

Appropriate dilutions were made with MeOH so that the final concentrations of Fos and Hct were 12.0 and 7.5 μg ml^{-1}, respectively, for derivative spectrophotometric measurements. Fourth derivative spectra of the solutions were recorded against MeOH. Derivative absorbance (D) values of the spectra at 217.7 and 227.9 nm were measured for the determination of Fos and Hct, respectively.

For HPLC measurements appropriate dilutions were made with 10 mM H_3PO_4/CH_3CN (1:1, v/v) so that the final concentrations of Fos and Hct and Ben, were 20.0, 12.5 and 15.0 μg ml^{-1}, respectively.

The quantity of the drugs were calculated using the regression equations of the corresponding calibration curves constructed for both the methods.

2.6. Assay validation

Synthetic mixtures prepared by adding known amounts of Fos and Hct were analyzed by both developed methods using the procedure outlined above. The mean percentage recoveries and relative standard deviations (R.S.D.) were calculated.

3. Results and discussion

The determination of Fos in tablets combined with Hct is not possible by direct UV-absorption measurements due to spectral overlap, however, Hct can be analyzed (Fig. 1a). Magri et al. solved this problem by using a computational program QUEST [1]. In this study, derivative spectrophotometric method has been

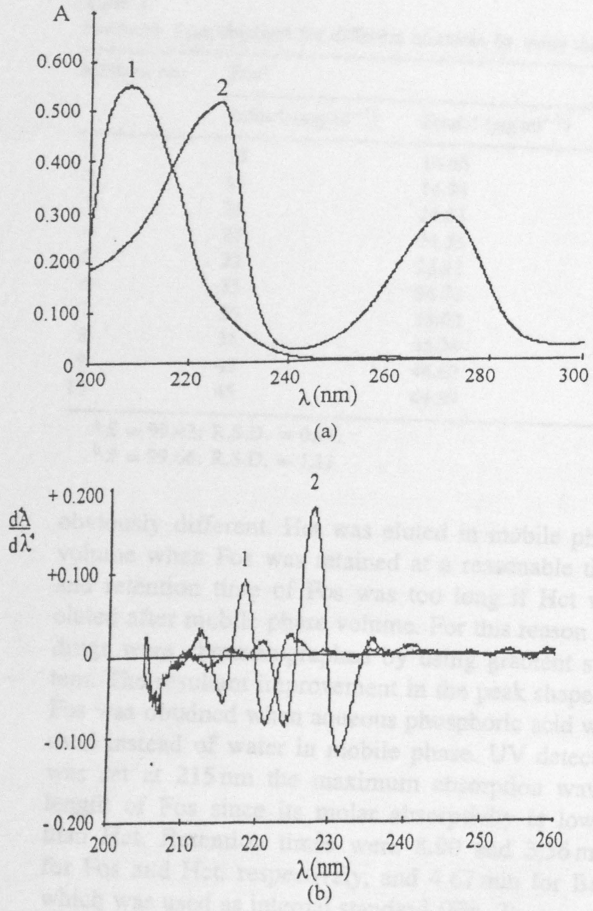

Fig. 1. (a) Zero-order absoption and (b) fourth derivative spectra of Fos (1) and Hct (2) (20 μg ml^{-1} of Fos and 4 μg ml^{-1} of Hct in methanol).

examined for the same purpose because it has been widely used for the determination of drugs especially in mixtures [16,17]. One to four derivative spectra of aqueous, methanolic and ethanolic solutions of Fos and Hct at appropriate concentrations were taken and observed. Fourth UV-derivative spectra of methanolic drug solutions were the best since the derivative absorbance peaks of both drugs were isolated in these spectra (Fig. 1b). Zero crossing wavelengths at 217.7 and 227.9 nm were selected since reproducible readings were obtained at these wavelengths. Other operating conditions were determined as mentioned in Section 2. Calibration curves were constructed by plotting $D = d^4A/d\lambda^4$ values at selected wavelengths against corresponding concentrations in the 5.0–45.0 μg ml^{-1} (Fos) and 0.5–9.0 μg ml^{-1} (Hct) concentration range. Regression equations of linear calibration graphs for Fos and Hct were calculated as $D = 0.00354C + 0.0041$ ($r = 0.9994$) and $D = 0.0365C + 0.00361$ ($r = 0.9996$), respectively.

As a second simultaneous analyse method for these drugs and to check the UV-derivative spectrophotometric results, a high-performance liquid chromatographic method was also developed. Optimum chromatographic conditions were examined to get a good separation. The mixtures of MeOH or CH_3CN and water at various ratio were tested as mobile phases on C-18 column by isocratic system. All of them were unsuccessful since the polarities of these drugs are

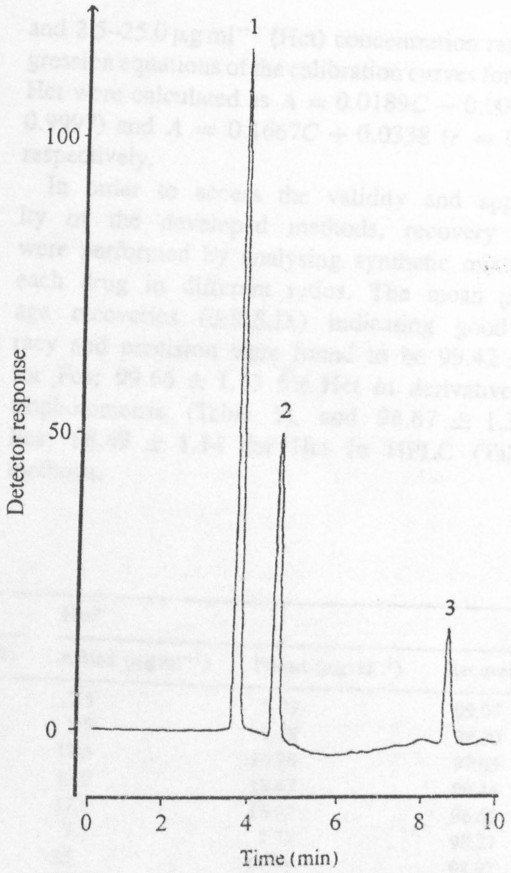

Fig. 2. Chromatogram obtained with the mixture of Hct (1), Ben (2) and Fos (3) (12.5 μg ml^{-1} of Hct, 15 μg ml^{-1} of Ben and 25 μg ml^{-1} of Fos in 10 mM H_3PO_4/CH_3CN, 1:1, v/v). Sample volume is 20 ml.

Table 1
Recovery data obtained for different mixtures by using the derivative method

Mixture no.	Fos[a]			Hct[b]		
	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%
1	15	14.66	97.73	1	0.97	97.00
2	15	14.94	99.60	5	5.02	100.40
3	25	24.83	99.32	3	3.00	100.00
4	25	24.55	98.20	7	7.05	100.71
5	25	25.11	100.44	9	8.91	99.00
6	35	34.72	99.20	1	0.997	99.70
7	35	35.00	100.00	5	5.00	100.00
8	35	35.28	100.80	9	8.89	98.78
9	45	44.60	99.11	3	3.02	100.67
10	45	44.89	99.76	7	7.02	100.29

[a] $\bar{x} = 99.42$; R.S.D. = 0.94.
[b] $\bar{x} = 99.66$; R.S.D. = 1.13.

obviously different. Hct was eluted in mobile phase volume when Fos was retained at a reasonable time and retention time of Fos was too long if Hct was eluted after mobile phase volume. For this reason the drugs were chromatographed by using gradient system. The resultant improvement in the peak shape of Fos was obtained when aqueous phosphoric acid was used instead of water in mobile phase. UV detector was set at 215 nm the maximum absorption wavelength of Fos since its molar absorptivity is lower than Hct. Retention times were 8.90 and 3.56 min for Fos and Hct, respectively, and 4.67 min for Ben which was used as internal standard (Fig. 2).

Peak area ratios (A/A_{is}) were plotted against corresponding concentrations in the 5.0–50.0 $\mu g\,ml^{-1}$ (Fos) and 2.5–25.0 $\mu g\,ml^{-1}$ (Hct) concentration range. Regression equations of the calibration curves for Fos and Hct were calculated as $A = 0.0189C - 0.0096$ ($r = 0.9997$) and $A = 0.1667C + 0.0338$ ($r = 0.9998$), respectively.

In order to access the validity and applicability of the developed methods, recovery studies were performed by analysing synthetic mixtures of each drug in different ratios. The mean percentage recoveries (±R.S.D.) indicating good accuracy and precision were found to be 99.42 ± 0.94 for Fos; 99.66 ± 1.13 for Hct in derivative spectrophotometric (Table 1), and 98.67 ± 1.35 for Fos; 98.49 ± 1.14 for Hct in HPLC (Table 2) methods.

Table 2
Recovery data obtained for different mixtures by using HPLC method

Mixture no.	Fos[a]			Hct[b]		
	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)
1	5	5.02	100.40	7.5	7.43	99.07
2	15	15.06	100.40	2.5	2.48	99.20
3	15	14.53	96.87	17.5	16.98	97.03
4	25	24.89	99.56	12.5	12.43	99.44
5	25	24.36	97.44	17.5	16.92	96.69
6	35	34.37	98.20	7.5	7.37	98.27
7	35	34.34	98.11	25	24.73	98.92
8	40	38.76	96.90	12.5	12.55	100.40
9	40	39.82	99.55	20	19.56	97.80
10	50	49.62	99.24	20	19.62	98.10

[a] $\bar{x} = 98.67$; R.S.D. = 1.35.
[b] $\bar{x} = 98.49$; R.S.D. = 1.14.

Table 3
Assay results of tablets containing 20 mg Fos and 12.5 mg Hct[a]

Compound	Statistical value	Derivative method	HPLC method
Fos	$\bar{x}$	19.73	19.85
	S.D.	0.35	0.57
	S.D. (%)	1.77	2.87
	t	0.44	0.44
	F	2.57	2.57
Hct	$\bar{x}$	12.07	12.24
	S.D.	0.20	0.34
	S.D. (%)	1.66	2.77
	t	1.05	1.05
	F	2.90	2.90

[a] $n = 6$; $P = 0.05$; $t = 2.23$; $F = 5.05$.

The proposed methods were applied to the determination of Fos and Hct in tablets and the results were statistically compared with each other at the 95% confidence level with the aid of t- and F-tests. As it can be seen from the Table 3, there is no significant difference between the two methods with the respect to mean values and standard deviations, since the calculated t- and F-values were less than the corresponding theoretical ones.

4. Conclusion

The methods developed in this study are widely used for the determination of drugs in pharmacopeas in contrary to the multiwavelength spectrophotometric method and these are easily carried out for simultaneous determination of Fos and Hct as well. It does not need any special equipment for derivative absorbance measuring in derivative spectrophotometric method.

Sensitivities of the both methods are the same (5 $\mu g\,ml^{-1}$) for Fos, whereas derivative spectrophotometric method (0.5 $\mu g\,ml^{-1}$) is five times more sensitive than HPLC (2.5 $\mu g\,ml^{-1}$) for Hct. These methods could not be compared with multiwavelength spectrophotometric method in terms of sensitivity since the range of working concentrations of the drugs were not clearly given in [1]. On the other hand, there is no difference between the precision of both the methods developed in this study (Table 1) as mentioned above, but multiwavelength spectrophotometric method is much more precise since the R.S.D. values were reported as 0.02 and 0.03 for Fos and Hct, respectively [1].

After the extraction of the drugs from tablet powder only a few minutes is required in both spectrophotometric methods. Although HPLC method is in need of longer time (10 min run time, 10 min waiting time) than the others, its high separation power may be an advantage for the analysis of these drugs in biological materials. The applicability of HPLC method to serum and urine samples is under investigated.

As a result, derivative spectrophotometric method is proposed for simultaneous determination of Fos and Hct, especially for routine quality controls. This method can be used for the rapid, reliable and sensitive quantitation of Fos and Hct in tablets.

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References

[1] A.L. Magri, F. Balestrieri, A.D. Magri, D. Marini, Talanta 42 (1995) 1719.
[2] J. Kirschbaum, J. Noroski, A. Cosey, D. Mayo, J. Adamovics, J. Chromatogr. 507 (1990) 165.
[3] X. Chen, Zhongguo Yaoxue Zazhi 25 (9) (1990) 543.
[4] A.F. Youssef, S.R. El-Shabouri, A.M.I. Mohamed, I. Maboud, Bull. Fac. Sci. 21 (1) (1992) 53.
[5] C.S.P. Sastry, M.V. Suryanarayana, A.S.R.P. Tipirneni, Talanta 36 (4) (1989) 491.
[6] Y. Long, J. Feng, S. Tong, Fenxi Huaxue 21 (8) (1993) 953.
[7] M. Schaefer, H.E. Geissler, E. Mutschler, J. Chromatogr. 143 (1977) 615.
[8] C. Fagerlund, P. Hartvig, B. Lindstroem, J. Chromatogr. 168 (1979) 107.
[9] W.J.A. Vandenheuvel, V.F. Gruber, R.W. Walker, F.J. Wolf, J. Pharm. Sci. 64 (1975) 1309.
[10] R. Weinberger, T. Pietrantonio, Anal. Chim. Acta 146 (1983) 219.
[11] J.J. Fett, F. Hischak, L.J.C.C. Love, Biomed. Chromatogr. 5 (1) (1991) 14.
[12] M. Yamazaki, Y. Ito, T. Suzuka, H. Yaginuma, S. Itoh, A. Kamada, Y. Orita, H. Nakahama, T.A. Nakanishi, A. Ando, Chem. Pharm. Bull. 32 (1984) 2387.
[13] R. Ruggieri, Boll. Chim. Farm. 99 (1960) 20.
[14] C. Van Kerchove, R. Bontemps, A. Schoenmakers, J. Pharm. Pharmacol. 34 (7) (1982) 420.
[15] Y.N. Liang, J.H. Sun, Fenxi Shiyanshi 13 (3) (1994) 57.
[16] B. Morelli, J. Pharm. Sci. 79 (1990) 261.
[17] M. Knochen, C. Altesor, I. Dol, Analyst 114 (1989) 1303.