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Liquid Chromatographic And Spectrophotometric Determination Of Triprolidine Hydrochloride, Acrivastine And Pseudoephedrine Hydrochloride In Pharmaceutical Preparations And Human Plasma

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ABSTRACT

The present work describes four new lc methods and four chemometric techniques in spectrophotometry for the analysis of pseudoephedrine hydrochloride (PSE) - triprolidin hydrochloride (TRP) and pseudoephedrine hydrochloride (PSE) – acrivastine (AC) combinations in pharmaceutical preparations and in human plasma. In LC methods, ACE C18 column with a mobile phase composed of methanol – phosphate buffer (pH:7) (80:20, v/v) was used for PSE – TRP combination in pharmaceutical preparations and human plasma, and for PSE - AC mixture, same column with a mobile phase composed of methanol – phosphate buffer (pH:7) (95:5, v/v) was used in pharmaceutical preparations and a mobile phase composed of 0.1 M $NaClO_4$ (pH:3)- acetonitril (95:5, v/v) by gradient elution technique was used in human plasma. Detection was at 220 nm for both combinations. Four chemometric techniques; CLS, ILS, PCR and PLS-1 methods were used for the spectrophotometric analysis of pharmaceutical formulations. In these techniques, the concentration data matrix were prepared by using the synthetic mixtures containing these drugs in 0.1M HCl for PSE – TRP mixture and in 0.1 M NaOH for PSE – AC mixtures. In the techniques, absorbance data matrix were obtained by the measurement of absorbances between 225.0 – 300.0 nm at 16 wavelengths in CLS, PCR and PLS–1 methods and between 245.0 – 300.0 nm at 12 wavelengths in ILS method in the zero-order absorption spectra of PSE – TRP mixture and between 240.0 – 285.0 nm at 19 wavelengths in the zero-order absorption spectra of PSE – AC combination in CLS, ILS , PCR and PLS–1 methods. The spectrophotometric procedures do not require any separation step. All the methods proposed were validated by analysing synthetic mixtures containing title drugs and they were successfully applied to the pharmaceutical formulations, capsule and tablet, and to human plasma and, the results were compared statistically with each other.

KEYWORDS

Pseudoephedrine hydrochloride; Triprolidine hydrochloride; Acrivastine; Chemometric methods; Pharmaceutical preparation; Human plasma; Liquid chromatography

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INTRODUCTION

Binary combinations of pseudoephedrine hydrochloride (PSE)–triprolidine hydrochloride (TRP) and pseudoephedrine hydrochloride (PSE)–acrivastine (AC) are frequently prescribed in medicine as anthistaminic drugs.

We used derivative and ratio spectra derivative spectrophotometry for the simultaneous spectrophotometric analysis of PSE – TRP and PSE – AC mixtures[1,4]. D.Deorsi et al.[2] used a HPLC method for the analysis of the mixture of triprolidin, pseudoephedrine, paracetamol and dextromethorphan, and T.G.Altuntas et al.[5] used a RP- HPLC method for the analysis of PSE – AC mixture. P.J.Gemperline et al.[3] has used background correction in multicomponent spectroscopic analysis using target transformation factor analysis for simultaneous determination of PSE and TRP in their binary mixture.

Except that the chemometric study made by P.J. Gemperline *et al.*[3] for PSE –TRP mixture which is very complicated and needs very sophisticated computer programme and mathemathical procedures, there is no chemometric technique used for the analysis of PSE–TRP and PSE–AC mixtures in the literatures. We wanted to applied classical chemometric methods for these combinations for easy analysis. Also, we didn't found any LC method in the literatures for the analysis of PSE–TRP and PSE–AC mixtures in human plasma. In addition, we wanted to develop new LC methods with the lower LOQ values compared to previous methods applied to the analysis of PSE–TRP and PSE–AC mixtures.

EXPERIMENTAL

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

In chemometric techniques, original spectra of the solution of PSE and TRP in 0.1M HCl in 225 – 300 nm range and original spectra of the solution of PSE and AC in 0.1 M NaOH in 240 – 285 nm range were used.

For HPLC, HP 1100 model liquid chromatograph was equipped with a model series of 613 22A degasser, 613 11A quaternary pump and 613 28A injector. The chromatograms were recorded and the peaks were quantitated using its automatic integrator. The separations were carried out in pharmaceutical formulations at ambient temperature on ACE C18 Column of 250 x 4.6 mm (5 μm particle size) and the mobile phase was methanol–phosphate buffer (pH:7) (80:20, v/v) for PSE–TRP combination and methanol–phosphate buffer (pH:7) (95:5) for PSE-AC combination. The flow rate was set at 1 mL/min with 20 μl as injection volume and the detection was at 220 nm for both combinations. As internal standard; paracetamol was used in the analysis of PSE–TRP combination and triprolidine hydrochloride was used in the analysis of PSE–AC combination. In human plasma; ACE C18 Column of 250 x 4.6 mm (5 μm particle size) with the mobile phase consisted of methanol – phosphate buffer (pH:7) (80:20, v/v) for PSE – TRP combination and ACE C8 column of 250 x 4.6 mm (5 μm particle size) with a mobile phase composed of 0.1M $NaClO_4$ (pH was adjusted to 3 with $HClO_4$): acetonitril (95:5, v/v) by gradient elution technique was used for PSE - AC mixtures. As internal standard; lidocaine hydrochloride was choosen in the analysis of both PSE–TRP and PSE–AC combinations. The flow rate was set at 1 mL/min with 20 μl as injection volume for PSE–TRP and the flow rate was 1.2 mL/min with 20 μl as injection volume for PSE–AC and the detection was at 220 nm for both mixture in human plasma analysis.

Computer software and hardware

In chemometric procedures, *Matlab 6.2* and *Minitab 12.2* softwares were used and run on PC Pentium III , 128 MB RAM, 1500 MHz computer.

Materials

Pseudoephedrine hydrochloride, triprolidine hydrochloride and acrivastine were kindly donated by GlaxoSmithKline, Turkey and they were used without further purification.

All the materials used in the spectrophotometric

analysis were of analytical reagent grade. HPLC grade solvents were used in LC procedures.

Standard solutions

Solutions of 200 mg/100 mL of PSE, 20 mg/ 100 mL TRP and 20 mg/100 mL AC were prepared in methanol in LC for pharmaceutical preparations and 100 mg/100 mL PSE, 20 mg/ 100 mL TRP were prepared in methanol, 100 mg/ 100 mL PSE and 20 mg/ 100 mL AC were prepared in 0.1M $NaClO_4$ – acetonitrile (1:1 v/v) in human plasma analysis. Solutions of 200 mg /100 mL of PSE and 20 mg /100 mL AC in 0.1 M NaOH and 20 mg/100 mL of PSE and 20 mg/100 mL TRP were prepared in 0.1 M HCl for spectrophotometric methods.

Solution of 10 mg/100 mL paracetamol was prepared in methanol as internal standard for the analysis of PSE – TRP combination, solution of 20 mg / 100 mL TRP was prepared in methanol as internal standard for the analysis of PSE–AC combination in pharmaceutical preparations. 10 mg/100 mL lidocaine hydrochloride was prepared as internal standard in methanol and 0.1M $NaClO_4$–acetonitrile (1:1 v/v) separately in human plasma analysis.

Sample preparation

1. In LC methods for pharmaceutical formulations: The content of 20 capsules or tablets were accurately weighed and powdered separately in a mortar. An amount of mass equivalent to one capsule or tablet content was dissolved in 100 ml of solution of methanol separately. After 20 min. of mechanically shaking and 15 min. of standing in the dark. Then, the solutions were filtered through 4.5 μm milipore filter in a 100 ml volumetric flasks separately. The residues were washed three times with 20 mL of solvents and the volumes were completed to the mark (I). After the addition of 7.4 mL of paracetamol as internal standard for the analysis of PSE – TRP combination, I was diluted 2/5 with the same solvents and 0.75 mL of triprolidine hydrochloride as internal standard was added for the analysis of PSE – AC combination before the completion. These solutions were injected separately to the column selected. The peak areas were measured for the determination of PSE, TRP and AC by using its integrator .

2. In LC methods for human plasma: 2 mL aliquot of plasma sample and 1 ml lidocaine hydrochloride (internal standard) solution was pipetted into a tube. Then, 2 mL methanol and 4 mL acetonitrile were added to precipitate the proteins in the sample. The samples were vortex-mixed and shaken vigorously for 5 min and centrifuged at 4000 rpm for 20 min. The supernatant was transferred to a clean tube and 20 μl was injected onto the LC system.

3. In the application of chemometric techniques: 20 tablets or capsules were accurately weighed and powdered in a mortar for two commercial preparations. The amount of the tablet mass equivalent to one tablet contents of each were dissolved in 60 mL of solvents proposed (0.1 M HCl for PSE–TRP mixture and 0.1 M NaOH for PSE–AC mixture). After 20 min. of mechanically shaking the solutions were filtered in a 100 mL volumetric flask. The residue was washed three times with 10 mL of solvents then the volume was completed to 100 ml with the same solvents (II). II was used without any dilution for the analysis of TRP – PSE mixture. II was diluted 1/6 with 0.1 M NaOH for the analysis of PSE - AC mixture.

Commercial pharmaceutical preparations

Actifed® (2.5 mg triprolidine hydrochloride and 60 mg pseudoephedrine hydrochloride/tablet) GlaxoSmithKlein Pharm.Ind., Turkey (batch no: 9524 HOA) and Duact® (8 mg acrivastine and 60 mg pseudoephedrine hydrochloride/capsule) GlaxoSmithKlein Pharm.Ind., Turkey (batch no: 9525 HOA) were assayed.

RESULTS AND DISCUSSION

LC methods

1. In pharmaceutical preparations

The developed HPLC methods have been applied for the simultaneous determination PSE - TRP and PSE-AC in their binary mixtures. Various mobile phases were assayed and methanol–phosphate buffer (pH:7) (80:20, v/v) mixture was found optimum for the good separation for PSE and TRP and

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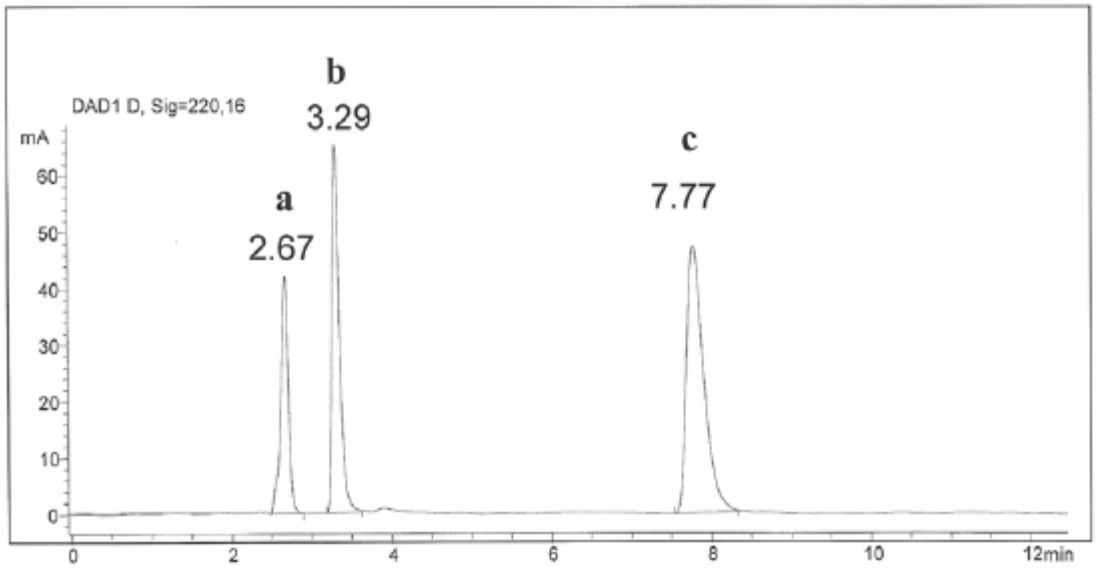

Figure 1: Typical chromatogram of a) internal standard (paracetamol), b) pseudoephedrine hydrochloride and, c) triprolidine hydrochloride in LC method for pharmaceutical preparations

TABLE 1: Training set used in PCR and PLS–1 techniques

Mixture no	PSE+AC		PSE+TRP	
	PSE µg/mL	AC µg/mL	PSE µg/mL	TRP µg/mL
1	1200	2	360	35
2	560	8	500	10
3	800	10	600	30
4	560	14	720	20
5	80	0	0	25
6	160	16	960	15
7	0	10	800	12
8	240	12	560	25
9	960	8	200	0
10	1440	2	160	50

methanol–phosphate buffer (pH:7) (95:5, v/v) mixture was found for PSE and AC on ACE C18 column. Quantitation of PSE, TRP and AC were made with UV detection at 220 nm. Retention times for PSE, TRP and internal standard (paracetamol) were found 3.29, 7.77 and 2.67 min respectively for ten replicates for PSE-TRP combination and 3.08, 2.20, 3.95 min for PSE, AC and internal standard (triprolidine hydrochloride) respectively for PSE-AC combination. Typical chromatograms of the drugs and internal standard were illustrated in figure 1 and 2. Peak areas were used in the quantitation procedures. Regression equations were:

a) in PSE-TRP mixture:

$y = 5.7561 x - 0.1288$ ($r = 0.9992$) for TRP and

$y = 0.0558 x + 0.9525$ ($r = 0.9993$) for PSE ,

where x is the concentration in µg/mL and y is the ratio of the areas of drug/internal standard,

b) in PSE-AC mixture:

$y = 0.0457 x + 0.2922$ ($r = 0.9990$) for PSE and

$y = 0.2267 x + 0.1835$ ($r = 0.9999$) for AC,

where x is the concentration in µg/mL and y is the ratio of the areas of drug/internal standard,

Linearity range was found 2 – 800 µg/mL for PSE and 1.0 – 60 µg/mL for TRP in PSE-TRP combination and, 0.8 - 400 g/mL for PSE and 0.4 - 100 µg/mL for AC in PSE -AC combination. LOQ was found 2 µg/mL for PSE and 1.0 µg/mL TRP and, 0.8 µg/mL for PSE and 0.4 µg/mL for AC for their binary mixtures in the methods.

LC method applied to the analysis of PSE-AC mixture with its internal standard (TRP) can also be used for the analysis of PSE-TRP mixture with the internal standard AC.

In the methods, the mean recoveries ± confidence interval (calculated as $x \pm t.SD/\sqrt{n}$ where x is the mean value, n is number of experiment and rela-

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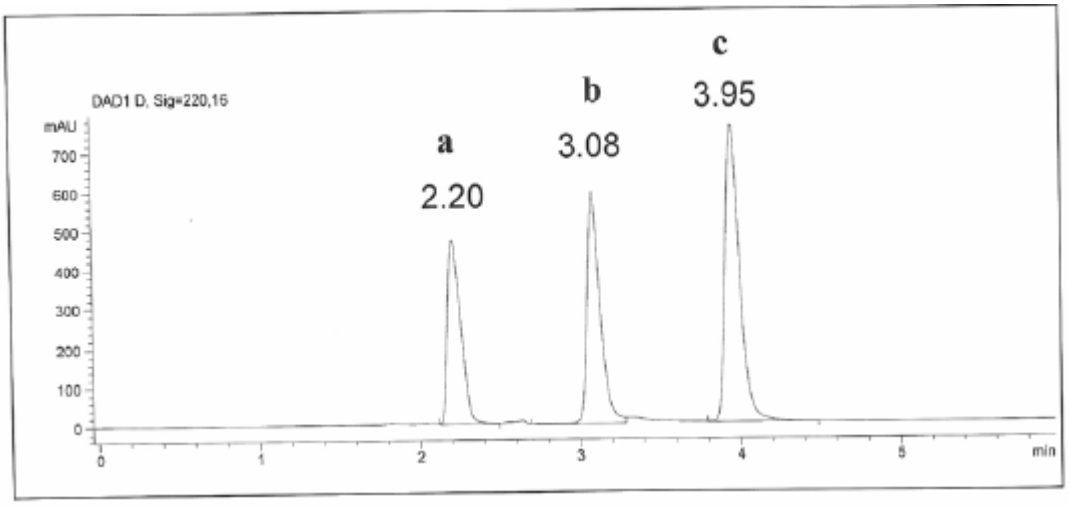

Figure 2: Typical chromatogram of a) acrivastine, b) pseudoephedrine hydrochloride and c) internal standard (triprolidine hydrochloride) in LC method for pharmaceutical preparations

TABLE 2: Recovery results for PSE and TRP in synthetic mixtures by the techniques proposed for pharmaceutical preparations

	CLS		ILS		PCR		PLS-1		LC	
	n=10		n=10		n=10		n=10		n=10	
	PSE	TRP	PSE	TRP	PSE	TRP	PSE	TRP	PSE	TRP
Mean recovery %	100.2	100.0	100.1	100.1	100.0	100.1	100.0	100.1	97.0	102.5
(±CI for P=0.05)	(± 0.55)	(± 0.69)	(± 0.16)	(± 0.40)	(± 0.40)	(± 0.76)	(± 0.40)	(± 0.73)	(± 0.56)	(± 2.33)
RSD %	1.12	1.47	0.34	0.85	0.84	1.59	0.84	1.57	0.61	2.48

***CI=confidence interval **n= number of sample**

TABLE 3: Recovery results for PSE and AC in synthetic mixtures by the techniques proposed for pharmaceutical preparations

	CLS		ILS		PCR		PLS-1		LC	
	n=10		n=10		n=10		n=10		n=10	
	PSE	AC	PSE	AC	PSE	AC	PSE	AC	PSE	AC
Mean recovery %	99.6	99.6	99.7	99.4	99.8	99.1	99.9	99.8	100.0	99.0
(±CI for P=0.05)	(± 0.52)	(± 0.47)	(± 0.40)	(± 0.72)	(± 0.35)	(± 0.38)	(± 0.77)	(± 0.55)	(± 0.37)	(± 2.15)
RSD %	1.17	1.07	0.89	0.89	0.78	0.87	1.74	1.25	0.27	1.59

tive standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in TABLE 2 and 3. Mean recoveries and relative standard deviations of the methods were found satisfactory.

2. In human plasma

For PSE–AC combination : We used gradient elution method for the analysis. In the method; initial mobile phase was acetonitrile – water containing 0.1M $NaClO_4$ adjusted to pH:3.0 with perchloric acid (5:95, v/v), then a linear gradient up to (67:33, v/v) in 25 min. Separation and quantification of PSE and AC on ACE C8 column were made with UV detection at 220 nm. Under these conditions, PSE, AC and internal standard (lidocaine hydrochloride) peaks were well resolved and their retention times were found 13.41, 22.49 and 17.12 min respectively. Typical chromatogram of the drugs and internal standard were illustrated in figure 3. Endogenous plasma components did not give any interfering peaks. Figure 4. shows typical chromatogram of blank plasma

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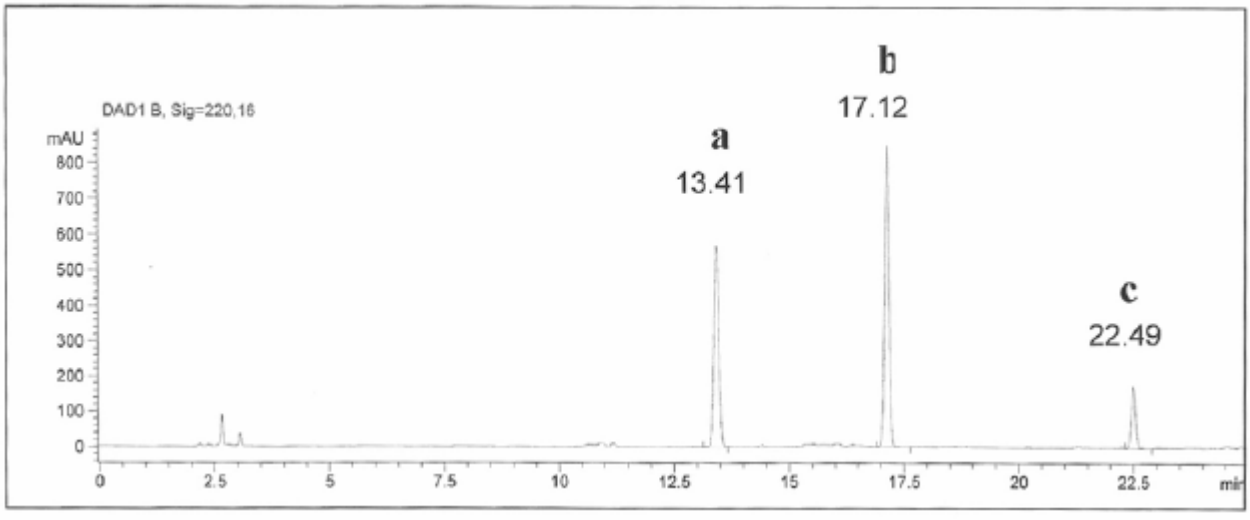

Figure 3: Typical chromatogram of a) pseudoephedrine hydrochloride, b) internal standard (lidocaine hydrochloride) and, c) acrivastine in LC method for human plasma

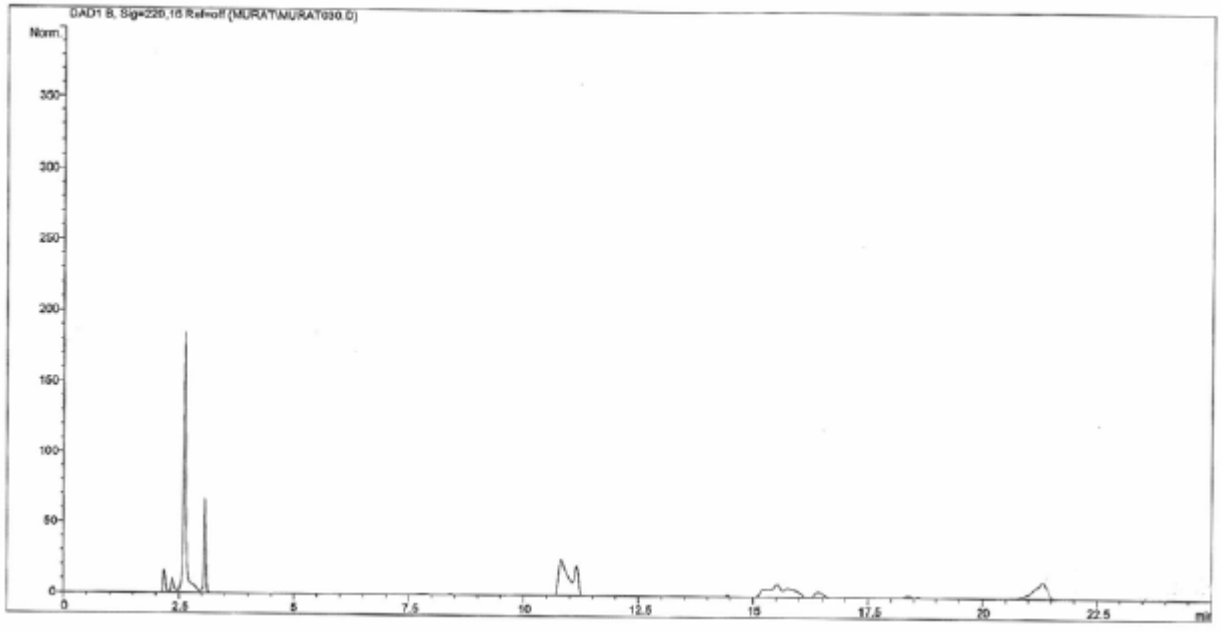

Figure 4: Typical chromatogram of drug–free human plasma for acrivastine–pseudoephedrine hydrochloride system

in comparison to spiked samples. Peak areas were used in the quantitation procedures.

Regression equations, calculated by adding known amounts of PSE and AC to drug-free plasma were as follows:

for PSE; y = 2,1275 x + 0.329 (r = 0.9992)

for AC y = 5.197 x – 1.158 (r= 0.9996)

where x is the concentration in μg/mL and y is the ratio of the areas of drug/internal standard.

Linearity range was found 2.4 – 600 μg/mL for PSE and 0.6 - 100 μg/mL for AC in PSE - AC combination. LOQ was found 2.4 μg/mL for PSE and 0.6 μg/mL for AC in the method. The relative analytical recovery for plasma at ten different concentrations of PSE and AC were determined and mean recoveries and relative standard deviations of the

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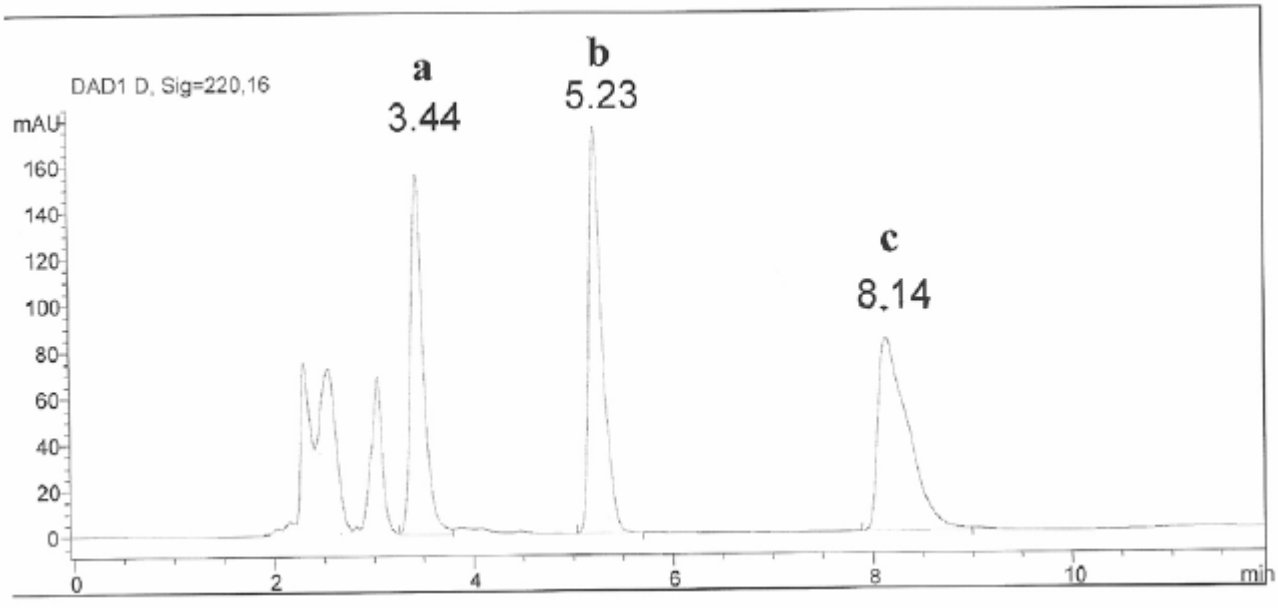

Figure 5: Typical chromatogram of a) pseudoephedrine hydrochloride, b) internal standard (lidocaine hydrochloride) and, c) triprolidine hydrochloride in LC method for human plasma

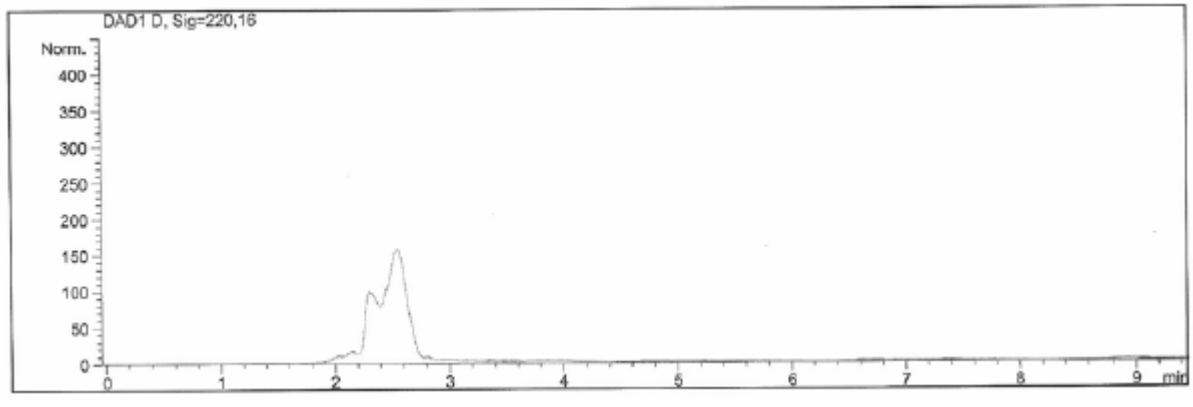

Figure 6: Typical chromatogram of drug–free human plasma for triprolidine hydrochloride – pseudoephedrine hydrochloride system

method were found as 98.6% and 0.62% for PSE and 98.4% and 1.62% for AC respectively in the method. For PSE–TRP combination : mobile phase consisted of methanol-phosphate buffer (pH:7) (80:20, v/v) mixture was found best separative solvent system for PSE and TRP on ACE C18 column. Quantitation of PSE and TRP were made with UV detection at 220 nm. Under these conditions, PSE, internal standard (lidocaine hydrochloride) and TRP peaks were well resolved and their retention times were found 3.44, 5.23 and 8.14 min respectively. Typical chromatogram of the drugs and internal standard were illustrated in figure 5. Endogenous plasma components did not give any interfering peaks. Figure 6 shows typical chromatogram of blank plasma in comparison to spiked samples. Peak areas were used in the quantitation procedures.

Regression equations, calculated by adding known amounts of PSE and TRP to drug-free plasma were as follows:

for PSE; y = y = 2,1733 x + 1,5137 (r = 0.9999)

for TRP y = 2,8414 x – 0,5657 (r = 0.9998)

where x is the concentration in μg/mL and y is the ratio of the areas of drug/internal standard.

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Linearity range was found as 1.0–800 μg/mL for PSE and 0.8-60 μg/mL for TRP in PSE-TRP combination. LOQ was found 1.0 μg/mL for PSE and 0.8 μg/mL for TRP for their binary mixtures in the methods. The relative analytical recovery for plasma at ten different concentrations of PSE and TRP were determined and mean recoveries and relative standard deviatons of the methods were % 100.74 and 2.10 % for PSE and % 100.1 % and 2.40 % for TRP respectively in the methods.

Chemometric methods

Figure 7 and 8 show the zero-order absorption spectra for PSE and TRP, and their binary mixture in 0.1 M HCl and, PSE and AC, and their binary mixture in 0.1 M NaOH. In the techniques, the absorbance data matrix were obtained by the measurements of absorbances between 225.0 – 300.0 nm in the intervals with $\Delta\lambda$ = 5 nm at 16 wavelengths in CLS, PCR and PLS–1 methods and between 245.0 – 300.0 nm with the $\Delta\lambda$ = 5 nm of intervals at 12 wavelengths in ILS method in the zero-order absorption spectra of PSE – TRP mixture and between 240.0 – 285.0 nm with the $\Delta\lambda$ = 2.5 nm of intervals at 19 wavelengths in the zero-order absorption spectra of PSE – AC combination for all four methods. Training set concentrations in PCR and PLS techniques were shown in TABLE 1 for these mixtures prepared for to avoid the co-linearity. In the techniques, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for prediction of the unknown concentrations of PSE, TRP and AC in their binary mixtures and pharmaceutical formulations.

Mean recoveries and relative standard deviations for the CLS, PCR, ILS and PLS–1 techniques for PSE–TRP mixture were found as 100.2% and 1.12%, 100.0% and 0.84%, 100.1% and 0.34, 100.0% and 0.84 % for PSE and 100.0 % and 1.47 %, 100.1% and 1.59%, 100.1% and 0.85%, 100.1% and 1.57% for TRP, respectively and for PSE – AC mixture were found 99.6% and 1.17%, 99.8% and 0.78%, 99.7% and 0.89, 99.9% and 1.74% for PSE and 99.6% and 1.07%, 99.1% and 0.87%, 99.4 % and 0.89 %, 99.8% and 1.25% for AC respectively in the synthetic mixtures of both drugs (TABLE 2 and 3).

The predictive ability of a model can be defined

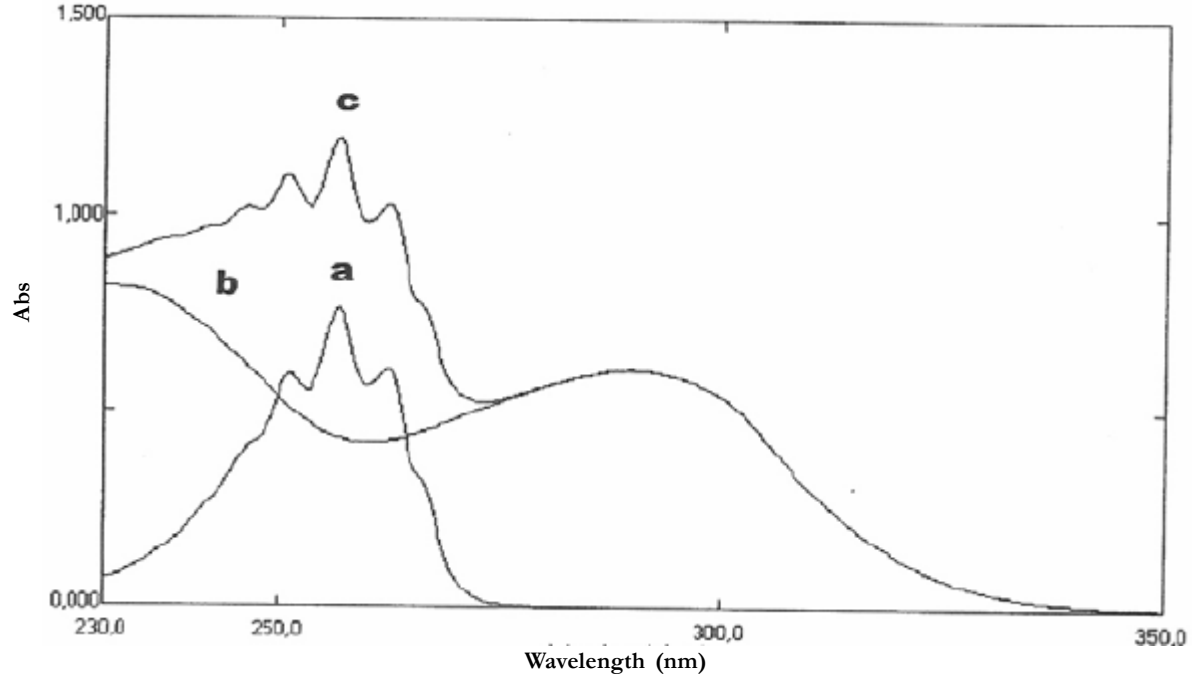

Figure 7: Zero-order absorption spectra of a) 960 μg/mL solution of pseudoephedrine hydrochloride, b) 20 μg/mL solution of triprolidine hydrochloride c) 960 μg/mL of pseudoephedrine hydrochloride + 20 μg/mL triprolidine hydrochloride mixture solution in 0.1 M HCl

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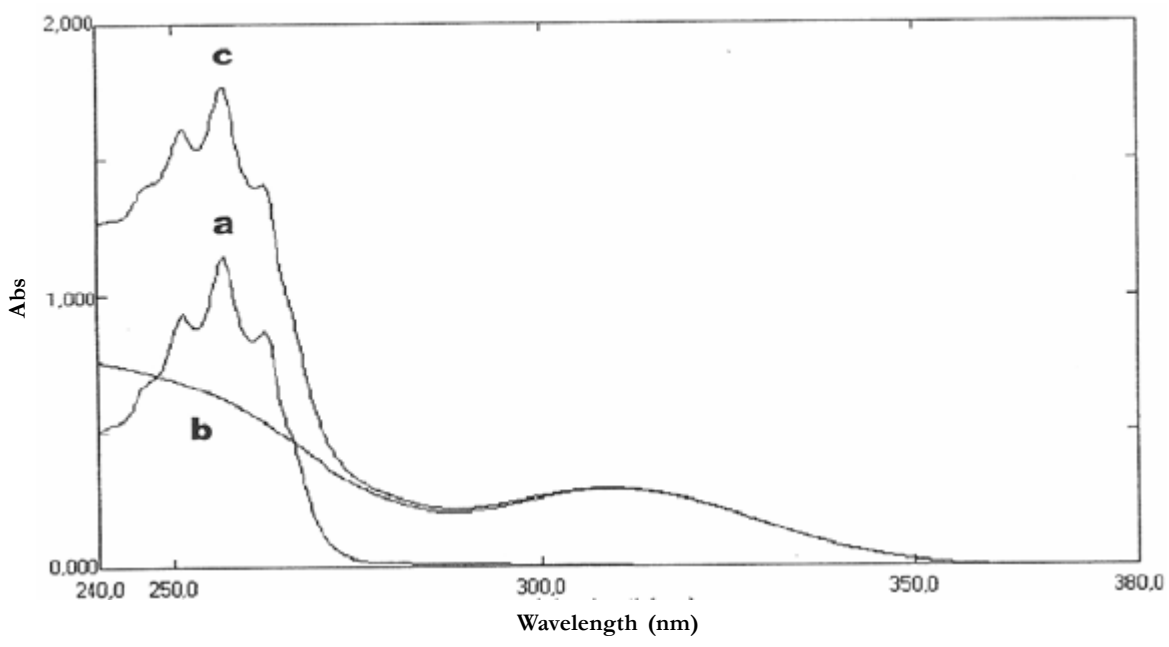

Figure 8: Zero-order absorption spectra of a) 1200 μg/mL solution of pseudoephedrine hydrochloride, b) 10 μg/mL solution of acrivastine c) 900 μg/mL of pseudoephedrine hydrochloride + 10 μg/mL of acrivastine mixture solution in 0.1 M NaOH

in various ways. The most general expression is the standard error of prediction (SEP) which is given the following equation:

$$\mathbf{SEP} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n}}$$

where C_i^{Added} is the added concentration of drug, C_i^{Found} is the predicted concentration of drug and *n* is the total number of synthetic mixtures.

In order to test the proposed techniques, the sets of synthetic mixtures containing the binary mixtures of drugs in variable composition were prepared. The results obtained in the application of CLS, PCR, ILS and PLS-1 methods to the same binary mixtures are indicated in TABLE 2,3. The standard error of prediction (SEP) were found completely acceptable (TABLE 4).

Another statistical value is the SEC (standard error of calibration) and the calculation of this value was realized by using following equation:

$$\mathbf{SEC} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n-p-1}}$$

where C_i^{Added} is the added concentration of drug, C_i^{Found} is the predicted concentration of drug and *n* is the total number of synthetic mixtures, p is the number of components in the mixtures.

The standard error of calibration (SEC) were also found acceptable in CLS, PCR, ILS and PLS methods in the synthetic mixtures containing these drugs in variable compositions prepared as indicated in TABLE 4.

In TABLE 4, r is defined as the correlation between constituent concentrations added and found, and shows the absorbance effects relating to the constituent of interest. r values obtained in the methods close to 1 mean no interference was coming from the other constituents in this set of synthetic mixtures.

Linearity range was 360-960 μg/mL for PSE and 5-50 μg/mL for TRP in PSE–TRP mixture and 80-1440 μg/mL for PSE and 2-16 μg/mL for AC in PSE–AC mixture in the methods.

LOD were found 36 μg/mL for PSE and 1 μg/mL for TRP in PSE-TRP mixture and 16 μg/mL for PSE and 0.4 μg/mL for AC in PSE–AC mixture, LOQ were found 360 μg/mL for PSE and 5 μg/mL

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TABLE 4: Summary of statistics in CLS, PCR , ILS and PLS-1 methods for the analysis of PSE-AC and PSE-TRP mixtures

				SEP					
Mixture	CLS	ILS	PCR	PLS-1	Mixture	CLS	ILS	PCR	PLS-1
PSE	5.94	2.05	5.27	5.13	PSE	8.10	8.10	7.98	8.47
TRP	0.38	0.17	0.41	0.40	AC	0.29	0.29	0.21	0.10
				SEC					
PSE	5.15	1.97	5.08	4.95	PSE	7.57	7.57	7.46	7.89
TRP	0.36	0.16	0.39	0.38	AC	0.28	0.28	0.19	0.09
				r					
PSE	0.9995	0.9999	0.9993	0.9994	PSE	0.9994	0.9994	0.9997	0.9997
TRP	0.9995	0.9999	0.9994	0.9994	AC	0.9990	0.9990	0.9990	0.9996
				Intercept					
PSE	14.5200	4.0579	0.9766	0.9447	PSE	-4.7600	-4.7600	-2.6800	-1.5313
TRP	0.0059	0.0403	0.0019	0.0019	AC	0.5100	0.5100	0.0860	0.0477
				Slope					
PSE	0.974	0.994	0.999	0.999	PSE	1.002	1.000	1.003	0.999
TRP	0.998	0.998	0.999	0.999	AC	0.930	0.930	0.980	0.999

for TRP in PSE–TRP mixture and 80 μg/mL for PSE and 2 μg/mL for AC in PSE–AC mixture in the methods proposed.

To select the number of factors, in order to model the system without overfitting the concentration data in the PLS-1 and PCR algorithms, a cross-validation method, leaving out one sample at a time was employed using training sets. In PLS-1 technique; three factors for both PSE, TRP and AC in PCR technique; three factors for both PSE, TRP and AC in PSE+TRP and PSE+AC mixture were found optimum for the determinations. We obtained the prediction error sum of squares (PRESS) and root-mean squares (RMS) minimum with these factors.

The numerical values were calculated by using softwares mentioned in materials section. Wold algorithm[6] was used in PCR calculations.

The numerical values were calculated by using softwares mentioned in experimental section.

Precision

The precision was determined by means of a one-way ANOVA including 10 replicates carried out on three successive days using four chemometric methods (CLS, ILS, PCR and PLS-1) and LC methods for synthetic mixtures of PSE-TRP and PSE-AC for pharmaceutical preparations. Snedecor *F* values below the tabulated levels were obtained in all cases (F=4.21, n_1=2, n_2=27), so there were no significant differences between the result obtained in the deter-

TABLE 5: Analysis of variance (ANOVA) for PSE–TRP combination for the proposed methods applied to pharmaceutical preparations

Parameters	Classical least squares CLS		Inverse least squares ILS		Principle component regression PCR		Partial least squares PLS-1		LC	
	PSE	TRP	PSE	TRP	PSE	TRP	PSE	TRP	PSE	TRP
Between-days variance	0.88	0.02	1.04	0.01	0.69	0.03	1.69	0.03	4.58	0.04
Within-days variance	2.63	0.03	2.84	0.01	2.08	0.03	2.20	0.05	2.69	0.22
F ratio	0.33	1.50	0.37	1.00	0.33	1.00	0.77	0.60	1.70	0.18
Mean value	500.9	10.0	500.3	10.0	499.2	10.0	500.3	10.0	160.3	10.0
Between-days RSD (%)	0.18	0.20	0.21	0.10	0.14	0.30	0.34	0.30	0.18	0.29
Within-days RSD (%)	0.53	0.30	0.11	0.10	0.42	0.30	0.09	0.50	0.50	0.28

Between-day and within-day degrees of freedom 2 and 27 respectively. The critical *F* ratio value for 2 and 27 degrees of freedom and a confidence level of 95 % is 4.21 .

TABLE 6: Analysis of variance (ANOVA) for PSE – AC combination for the proposed methods applied to pharmaceutical preparations

Parameters	Classical least squares CLS		Inverse least squares ILS		Principle component regression PCR		Partial least squares PLS-1		LC	
	PSE	AC	PSE	AC	PSE	AC	PSE	AC	PSE	AC
Between-days variance	2.36	0.10	2.18	0.07	2.51	0.03	2.08	0.03	2.89	0.04
Within-days variance	0.90	0.11	1.65	0.04	0.87	0.05	0.68	0.05	2.08	0.02
F ratio	2.62	0.91	1.32	1.75	2.88	0.60	3.06	0.60	1.39	0.20
Mean value	575.3	10.0	574.4	10.1	575.7	10.0	574.5	10.0	160.0	32.0
Between-days RSD (%)	0.76	1.00	0.73	0.70	0.78	0.30	0.71	0.30	0.77	0.06
Within-days RSD (%)	0.10	1.10	0.04	0.40	0.06	0.50	0.07	0.50	1.44	0.03

Between-day and within-day degrees of freedom 2 and 27 respectively. The critical *F* ratio value for 2 and 27 degrees of freedom and a confidence level of 95 % is 4.21

mination of each drug in the presence of other on different days (TABLE 5 and 6).

Robustness

The robustness of a method is its ability to remain unaffected by small change in methods. In LC method; three analytical columns and several batches of reagents were used to assess the robustness of the method. The method was not sensitive to small change in system parameters but has only been validated using a single analyst. Also changing the concentration of HCl and NaOH from 0.01 to 0.5 M did not effect the results in all the spectrophotometric methods.

Applications

Comparison of the spectra of PSE and TRP in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the zero-order spectra did not change and also after addition of known amount of these active ingredients to the commercial formulations powder were found the amount of these drugs did not change. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate) did not interfere the quantitation of PSE and TRP in the methods. Same observations were seen for PSE and AC in their analysis. All the results obtained by using the methods described above were compared with each other and no significant difference was observed between the amount of drugs found as theoretical values for *t* at P = 0.05 level for commercial formulation (TABLE 7 and 8).

Also, the results obtained using proposed chemometric methods for the determination of PSE, TRP and AC in their binary mixtures in the pharma-

TABLE 7: Assay results of commercial preparation (ACTIFED® tablet) (mg)

Methods	(Label claim = 60 mg/tablet) PSE		(Label claim= 2.5 mg/tablet) TRP	
	mean ± SD**	*t* values	mean ± SD	*t* values
Classical least squares CLS)	59.40 ± 0.82	CLS – ILS =0.14 CLS – PCR = 1.28 CLS – PLS = 1.82 ILS – PCR =0.25	2.54 ± 0.31	CLS – ILS = 0.67 CLS – PCR = 0.56 CLS – PLS = 2.13 ILS – PCR = 0.17
Inverse least squares (ILS)	59.48 ± 0.39	ILS – PLS = 0.25 PCR – PLS = 0.02 HPLC – CLS = 1.90	2.50 ± 0.05	ILS – PLS= 0.17 PCR – PLS = 0.13 HPLC – CLS = 1.26
Principal component regression (PCR)	59.36 ± 0.85	HPLC – ILS = 1.91 HPLC– PCR= 1.03 HPLC– PLS =2.00	2.51 ± 0.27	HPLC– ILS = 0.16 HPLC– PCR = 1.12 HPLC– PLS = 1.14
Partial least squares (PLS-1)	59.00 ± 0.85		2.51 ± 0.26	
LC	61.43 ± 0.37		2.50 ± 0.09	

***Obtained results are average of ten tablets for four techniques; **SD=standard deviation, ***Theoretical value for *t* at P : 0.05 level = 2.26**

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TABLE 8: Assay results of commercial preparation (DUACT® capsule) (mg)

	(Label claim= 60 mg/capsule) PSE		(Label claim= 8 mg/capsule) AC	
	mean ± SD**	*t* values	mean ± SD	*t* values
Classical least squares (CLS)	60.28 ± 1.29	CLS – ILS = 0.02 CLS – PCR = 1.88 CLS – PLS = 1.87 ILS – PCR = 1.89	7.93 ± 0.08	CLS – ILS =0.19 CLS – PCR = 0.36 CLS – PLS = 0.36 ILS – PCR = 0.18
Inverse least squares (ILS)	60.27 ± 1.29	ILS – PLS = 1.89 PCR – PLS = 0.02 HPLC – CLS = 2.02	7.94 ± 0.06	ILS – PLS= 0.18 PCR – PLS = 0.03 HPLC – CLS = 1.48
Principal component regression (PCR)	60.44 ± 1.22	HPLC – ILS = 2.04 HPLC– PCR = 2.07 HPLC– PLS = 1.66	7.96 ± 0.10	HPLC– ILS = 1.52 HPLC– PCR = 1.02 HPLC– PLS = 1.51
Partial least squares (PLS-1)	61.05 ± 1.22		7.94 ± 0.10	
LC	62.21 ± 1.16		8.18 ± 0.13	

***Obtained results are average of ten tablets for four techniques; **SD=standard deviation, ***Theoretical value for *t* at P : 0.05 level = 2.26**

ceutical preparations, capsule and tablet, selected was also compared with those obtained by the LC methods developed by us and no significant differences were observed statistically. Amounts in the assay using chemometric techniques were found in coincidence with the LC methods (TABLE 7 and 8).

CONCLUSION

In this study; two new HPLC methods and four chemometric techniques were developed for the simultaneous analysis of PSE-TRP and PSE-AC combinations in pharmaceutical formulations. The proposed chemometric techniques (CLS, ILS, PCR and PLS-1) could be applied with great success for the simultaneous determination of PSE, TRP and AC in their binary mixtures and in the pharmaceutical preparations. Satisfactory results were obtained by these chemometric methods but, they need softwares for the mathematical calculations. Using only zero-order spectra in the procedures and not need any other graphical mode, such as ratio mode in the instruments is the advantages for the chemometric methods when compared with derivative and ratio spectra derivative spectrophotometric methods[1,4] although the working range were found similar. By not needing any time consuming sample preparation procedures and using 0.1 M HCl and 0.1 M NaOH as solvent , spectrophotometric methods developed are easier and cheaper when compared with the HPLC methods. Also, LOQ values found in our HPLC methods are lower than that of indicated in all the literature HPLC methods[2,3,5].

Gemperline *et al.* have used target transformation principle component regression technique which is a modified application of component regression technique for the spectrophotometric analysis of PSE–TRP combination[3], but we used principle component regression (PCR) technique classically in addition to the other classical chemometric methods (CLS, ILS, PLS-1) which are inexist in the literatures and LOQ values found in our chemometric methods are lower than that of indicated by Gemperline *et al.* . Also, we have developed and validated four chemometric methods (CLS, ILS, PCR, PLS-1) for the analysis of PSE–AC combination which are inexist in the literatures. All the spectrophotometric methods proposed in this article were validated and compared with each other and with our LC methods. These four chemometric methods and two LC methods for each mixture were found suitable for simple and precise routine analysis of the pharmaceutical preparation selected. Good agreement was seen in the assay results of pharmaceutical preparations widely used in Turkey, capsule and tablet, for all the methods proposed in the text. As a new LC method for the analysis of PSE – TRP combination in human plasma, we used the LC method for the analysis of PSE – TRP mixture in pharmaceutical formulations developed by us but with different internal standard (lidocaine hydrochloride). Also, we

developed a new LC method for the analysis of PSE – AC in human plasma. Our LC method proposed for the analysis of PSE and TRP in human plasma can also be used for the analysis of these active ingredients in pharmaceutical preparations. But the LC method developed for the simultaneous determination of PSE and AC in pharmaceutical preparations can not be used for the determination of these drugs in human plasma due to the interference from plasma components. LC methods for human plasma are sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies.

REFERENCES

[1] E.Dinc, F.Onur; S.T.P.Pharma Sciences, **8(3)**, 203-208 **(1988)**.

[2] D.Deorsi, L.Gagliardi, A.Bolasco, D.Tonelli; Chromatographia, **43(9-10)**, 496-500 **(1996)**.

[3] P.J.Gemperline, S.E.Boyette, K.Tyndall; Applied Spectroscopy, **41(3)**, 454–459 **(1987)**.

[4] E.Dinc, F.Onur; Anaytical Letters, **30(6)**, 1179-1191 **(1997)**.

[5] T.G.Altuntas, S.S.Zanooz, D.Nebioglu; Journal of Pharmaceutical and Biomedical Analysis, **17(1)**, 103-1091 **(1998)**.

[6] H.Wold; 'Multivariate Analysis', P.R.Ed.Ademic, New York **(1966)**.