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Derivative Spectrophotometry Applied to the Determination of Triprolidine Hydrochloride and Pseudoephedrine Hydrochloride in Tablets and Dissolution Testing

L. Sriphong, A. Chaidedgumjorn, and K. Chaisuroj

***Abstract*—**A spectrophotometric method was developed for simultaneous quantification of pseudoephedrine hydrochloride (PSE) triprolidine hydrochloride (TRI) using second derivative method (zero-crossing technique). The second derivative amplitudes of PSE and TRI were measured at 271 and 321 nm, respectively. The calibration curves were linear in the range of 200 to 1,000 μg/ml for PSE and 10 to 50 μg/ml for TRI. The method was validated for specificity, accuracy, precision, limit of detection and limit of quantitation. The proposed method was applied to the assaying and dissolution of PSE and TRI in commercial tablets without any chemical separation. The results were compared with those obtained by the official USP31 method and statistical tests showed that there is no significant between the methods at 95% confidence level. The proposed method is simple, rapid and suitable for the routine quality control application.

***Keywords*—**Triprolidine, Pseudoephedrine, Derivative spectrophotometry, Dissolution testing.

I. Introduction

PSEUDOEPHEDRINE hydrochloride (PSE) is a sympathomimetic drug that has been for the relief of nasal decongestion [1] and tripolidine hydrochloride (TRI) is an antihistamine drug [2]. The combination of PSE and TRI were shown to make distinct contribution to the treatment of allergic rhinitis [3] and have been commonly used in respiratory combination products.

The United States Pharmacopoeia (USP31) method for quantification of PSE and TRI in combined formulations and dissolution test is a normal phase HPLC [4]. The methods for simultaneous determination of PSE and TRI in pharmaceutical formulations were reported including HPLC [5-6] and difference spectrophotometry [7]. There were derivative spectrophotometric methods published for the determination of PSE combined with ibuprofen, fexofenadine, cetirizine and loratadine in binary mixtures [8-9]. However, there is no derivative spectrophotometric method reported for simultaneous determination of PSE and TRI in dissolution testing.

Derivative spectrophotometry is a very useful analytical technique for determining binary and multicomponent mixtures of drugs with overlapped spectra. In this work, derivative spectrophotometric method was reported to accomplish the simultaneous determination of PSE and TRI without prior chemical separation. The aim of this work was to develop an alternative analytical method to the USP31 method which is time-consuming and need expensive reagents. The methods were applied to both tablet analysis and dissolution testing and compared with the official USP31 method.

L. Sriphong is with the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Sanam Chandra Palace, Nakhon Pathom 73000, Thailand (corresponding author to provide phone: +6634-255-800; fax: +6634-255-801; e-mail: lawan@ su.ac.th).

A. Chaidedgumjorn is with the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Sanam Chandra Palace, Nakhon Pathom 73000, Thailand (e-mail: author@lamar. colostate.edu).

K. Chaisuroj is with the Medicine Supply, 77/11 Thatamnak, Nakornchaisri, Nakhonpathom, 73120 Thailand. (e-mail: qw_wp@hotmail.com).

II. Experimental

A. Materials

Standard of PSE and TRI were obtained from Devision of Medical Sciences, Nonthaburi, Thailand. All reagents and chemicals used were analytical grade and procured from local sources. All pharmaceutical preparations were purchased from local market. They included Actifed® Nasolin® and Profed® tablets, claimed to contain 60 mg PSE and 2.5 mg TRI.

B. Instrumentation

Agilent 8453E Diode array Spectrophotometer (Agilent, USA) was used. The dissolution apparatus was a Varian Model VK 7000 (Varian, USA). The HPLC system consisted of an Agilent 1100 series pump, a solvent degasser, an autosampler, a photodiode-array detector (DAD) and Chemstation software Version A.08.01 (Agilent, USA).

C. Derivative Spectrophotometric Method

Different aliquots of PSE and TRI solution were prepared and diluted with 0.1 M HCl to produce concentrations ranging from 200 to 1000 μg/ml for PSE and 10 to 50 μg/ml for TRI. The second derivative spectra were recorded using the prepared solutions against 0.1 M HCl as blank. The values of the second derivative absorbance (^{2}D) for either compound were obtained using five different concentrations by

measuring each concentration against solvent blank at the chosen wavelength; 271 and 321 nm for PSE and TRI, respectively. The values of ^{2}D were plotted against corresponding concentrations to construct the calibration curves.

D. Method Validation

The developed methods were validated according to ICH guideline [10] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte.

E. USP31 Method

The chromatographic method based on HPLC separation using a normal-phase column (ZORBAX RX-SIL, 4.6 x 150 mm, 5 µm) with a mobile phase consisting of methanol and ammonium acetate solution (1 in 250) (17:3). The flow rate was 1.2 ml/min. UV detection was set at 254 nm. Inject volume for assay and dissolution testing were 10 and 50 µl, respectively. The quantities of PSE and TRI dissolved in comparison with a standard solution having known concentrations.

F. Analysis of PSE and TRI in Tablets

For Second Derivative Spectrophotometric Method

Ten tablets were finely powdered, weighed, a portion equivalent to 5 mg of TRI and 120 mg of PSE was sUSP31ended in 0.1 M HCl, transferred quantitatively into 50 ml volumetric flask, sonicate for 15 min, complete to volume with 0.1 M HCl, filter and then dilute 2.5 ml of filtrate to 10.0 ml. The solution was measured for the second derivative absorbance as described in sections II.C.

For USP31 Method

Ten tablets were finely powdered, weighed, a portion equivalent to 5 mg of TRI and 120 mg of PSE was suspended in the mobile phase, transferred quantitatively into 50 ml volumetric flask, sonicate for 15 min, complete to volume with the mobile phase filter and then dilute 1.0 ml of filtrate to 10.0 ml with mobile phase.

G. Dissolution Testing

Dissolution test of Profed® tablets were carried out in 45 min according to USP31 method [4], in 900 ml of water at 37.0±0.1 °C (n=6) in Apparatus 2 (paddle). The amount of drug dissolved was sampled, pooled (n=3) and analyzed by the derivative method presented in section II.C. and the USP31 method in section II.E. Amounts of the dissolved drugs were calculated in comparison with a standard solution having known concentrations. The obtained results from both methods were statistically compared by paired *t*-test at 95% confidence interval.

III. Results and Discussion

A. Method Development

The zero-order (^{0}D) spectrum TRI showed a marked overlapping with the spectrum of PSE (Fig. 1a), thus TRI interfered with the analysis of PSE while PSE did not interfere with the analysis of TRI at λ_{max} 290 nm. The zero-crossing points were assigned from the ^{1}D and ^{2}D spectra of PSE and TRI (Fig. 1b-1c). The ^{1}D and ^{2}D zero-crossing point of PSE and TRI were found at 252, 257 and 310 nm and 231 and 262 nm (Fig. 1b) and 256, 259 and 321 nm and 230, 249 and 271 nm (Fig. 1c), respectively.

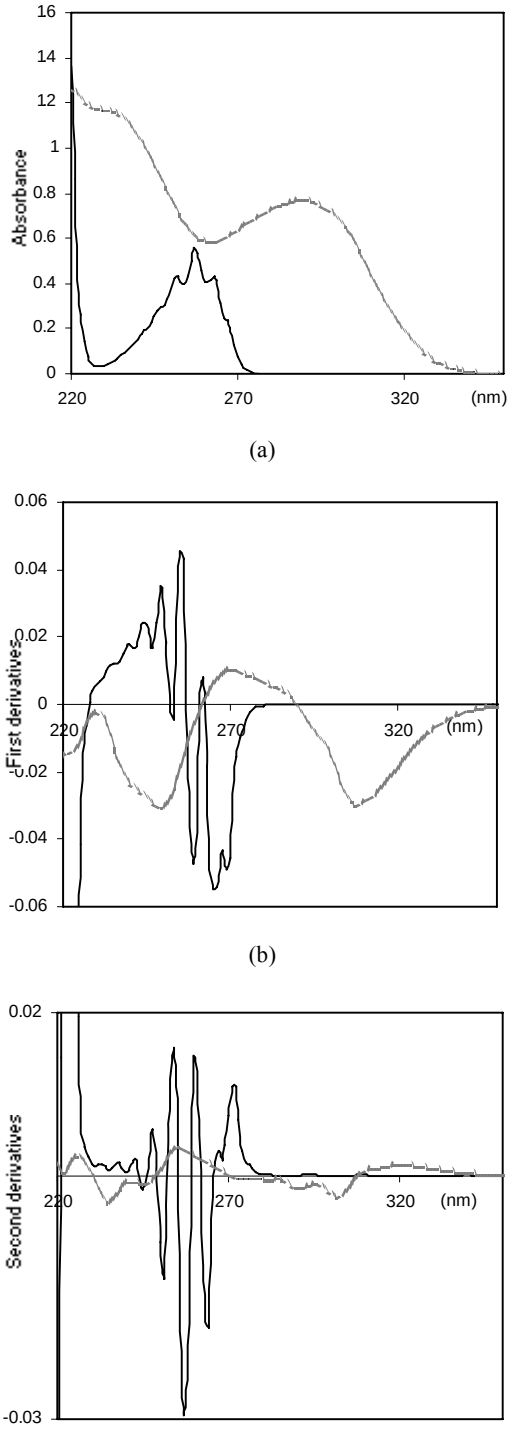

Fig. 1 The absorption spectra of PSE (600 µg/ml), (⎯⎯ line) and TRI (30 µg/ml), (---- line); (a) zero-order (b) first derivative and (c) second derivative

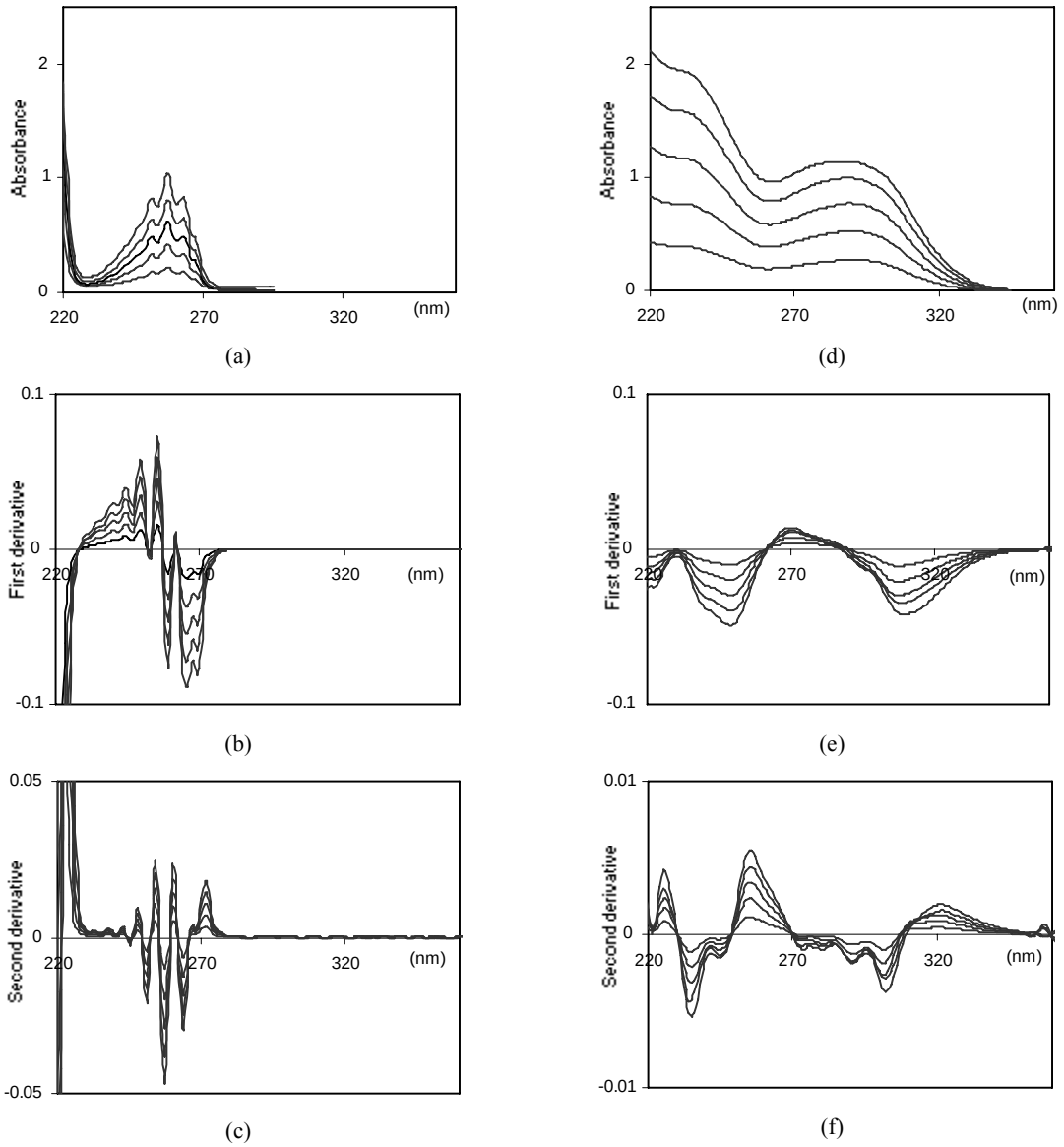

Fig. 2 Calibration curve of PSE 200-1,000 µg/ml; (a) zero-order, (b) first derivative and (c) second derivative. Calibration curve of TRI 10-50 µg/ml; (d) zero-order (e) first derivative and (f) second derivative

The selection of the optimal wavelength is based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration. The absorption spectra of PSE and TRI in the range of 200-1000 µg/ml and 10-50 µg/ml were measured (Fig. 2a-2f). The ^{2}D spectra showed better resolution and linearity of the calibration curve of PSE. Therefore, 271 nm (zero- crossing point of TRI) and 321 nm (zero amplitude of PSE) were chosen for the simultaneous determination of PSE and TRI in binary mixture, respectively.

Direct spectrophotometry (zero order) can also be used for the determination of TRI alone at 290 nm. A linear correlation was obtained between the absorbance at 290 nm and the corresponding concentration in the range of 10-50 µg/ml and found to be linear (y = 0.0218c - 0.0952, r^2= 0.9919). However, the determination of PSE in the presence of TRI was more accurate when using ^{2}D technique.

B. Method Validation

Specificity

The method specificity was assessed by comparing the spectra obtained from the commercial formulations and the synthetic mixture from standard solutions. The absorption spectra were similar (Fig. 3) revealed there was no interference from the excipients in the tablets.

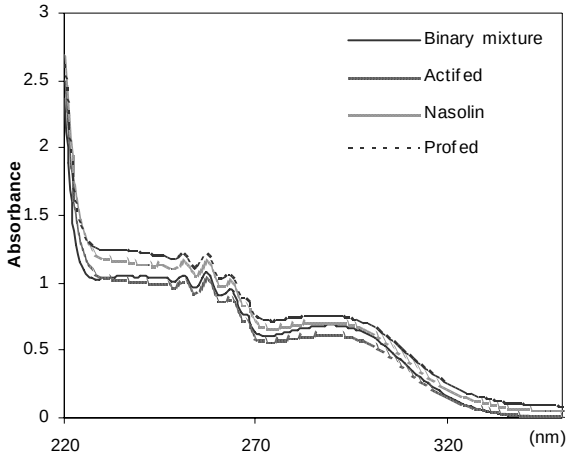

Fig. 3 The absorption spectra of binary mixture and of commercial sample solutions (Actifed®, Nasolin® and Profed®) contained of PSE 600 μg/ml and TRI 25 μg/ml

Linearity and Range

The range for PSE and TRI was optimized at 200–1000 and 10–50 μg/ml, respectively. The second derivative absorption of PSE and TRI were measured at 271 and 321 nm. The linearity was evaluated by the least square regression method. The correlation coefficient of the standard curves (n = 3) for all the two drugs was greater than 0.99. The mean values of correlation coefficient, slope and intercept were shown in Table I.

TABLE I
METHOD VALIDATION RESULTS

Parameter	PSE	TRI
Linearity range (μg/ml)	200-1,000	10-50
Regression equation (y)[a]		
Intercept (a)	-0.0008	0.0001
Slope(b)	0.00018	0.0004
Correlation coefficient	0.9995	0.9974
LOD (μg/ml)	17.6	1.2
LOQ (μg/ml)	53.2	3.8
Precision (%RSD)		
Repeatability (*n*=7)	0.33	0.78
Intermediate precision (*n*=9)	4.14	1.93
Accuracy		
% recovery (*n*=9)	98.1	102.2
% RSD	0.66	2.24
Specificity	no interference	no interference

[a] Y = a + bc, where c is the concentration of drug in and Y is the amplitude at the specified wavelength in derivative spectrophotometry.

Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and the RSD was calculated. The mean % recoveries of PSE and TRI was found to be 98.1% and 102.2%, respectively (Table I).

Precision

The repeatability of the method was determined by assaying seven standard solutions of PSE and TRI at the concentration 20 and 400 μg/ml, respectively. The RSD of repeatability was 0.33% and 0.78% for PSE and TRI, respectively (Table I). Intermediate precision was determined by the inter-day assay of three level 80%, 100% and 120% of label claimed in triplicate. The RSD for inter-day analysis was found to be 4.14% and 1.93% for PSE and TRI, respectively (Table I).

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ of PSE and TRI were calculated as 3.3σ/S and 10σ/S where σ is the standard deviation of the intercept and S is the slope of the calibration curves (n=3). The data were presented in Table I.

C. Applications

The USP31 method is not only time-consuming but also organic solvent-consuming because the normal phase HPLC system needed many organic solvents (hexane, isopropanol and methanol) for washing the column. While the developed derivative spectrophotometric method is rapid and convenient according to analyzing without any chemical separation and preparation of mobile phase. This indicated the superiority of the simple spectrophotometric method over the HPLC procedure. Moreover, no use of organic solvent can reduce the cost of analysis.

The content of PSE and TRI in samples of the commercial tablets were analyzed by the proposed spectrophotometric and USP3131 method (Table II). A paired t-test was applied for the comparison between the results from these two methods at 95% confidence interval. The calculated *t* value did not exceed the corresponding two-tail critical value (Table 2), suggesting that there was no significant difference (P>0.05) between the mean contents of PSE and TRI assayed by UV and USP31 method.

For dissolution testing, the method was modified by measuring the zero order absorbance of TRI at 290 nm instead of the ^{2}D amplitudes of TRI at 321 nm which was very low. The amount dissolved of PSE and TRI determined by the spectrophotometry and USP31 method were compared statistically at 95% confidence interval (Table III). The data showed that there was no significant difference (P>0.05) between the results obtained from this method in comparison with the USP31 method since the calculated *t* value was lower than the corresponding two-tail critical value. Both met the USP31 tolerances criteria for PSE and TRI dissolution which is not less than 75% of the labeled amounts, dissolved in 45 minutes.

TABLE II
DETERMINATION OF PSE AND TRI IN COMMERCIAL TABLETS BY DERIVATIVE SPECTROPHOTOMETRY AND USP31 METHOD

	% declared±SD (PSE)		% declared±SD (TRI)	
Method	$^2D_{271\ nm}$	USP31	$^2D_{321\ nm}$	USP31
Actifed®	99.6±2.08	100.7±0.65	99.9±2.88	95.9±0.91
Nasolin®	104.8±2.47	103.2±0.56	98.8±0.23	101.6±0.85
Profed®	100.7±0.19	102.6±0.78	100.1±1.59	101.1±0.96
Average±SD	101.7±2.74	102.2±1.29	98.7±2.15	100.5±1.40
t-Test: paired two sample of means				
t Stat	-.450		-3.598	
t Critical	4.303		4.303	

IV. CONCLUSION

A derivative spectrophotometric method was developed and showed the advantages of being simple, rapid, cost effective and time saving. Statistical tests confirmed the method was

comparable to the USP31 method and can be employed as an alternative analytical method for simultaneous determination of these two drugs. Therefore, the method is suggested for routine analysis of PSE and TRI in tablet dosage form and dissolution testing.

TABLE III
DISSOLUTION TESTING OF PSE AND TRI IN PROFED® TABLETS BY SPECTROPHOTOMETRY AND USP31 METHOD

	% dissolved (PSE)		% dissolved (TRI)	
Method	$^2D_{271\ nm}$	USP31	$A_{290\ nm}$	USP31
Tablet 1	101.5	100.2	123.8	123.5
Tablet 2	93.5	96.6	99.5	96.2
Tablet 3	101.5	100.4	99.1	87.2
Tablet 4	93.5	98.8	91.1	109.7
Tablet 5	101.5	100.8	93.0	91.6
Tablet 6	101.5	101.9	103.1	109.9
Average	98.8	99.8	101.6	103.0
SD	4.14	11.76	1.87	13.67
t-Test: paired two sample of means				
t Stat	-.639		-.692	
t Critical	2.571		2.571	
Method	$^2D_{271\ nm}$	USP31	$A_{290\ nm}$	USP31
Pooled 1	101.5	100.8	94.6	88.9
Pooled 2	93.5	101.0	99.1	102.0
Pooled 3	101.5	100.3	102.3	98.8
Average	98.8	100.7	98.7	96.4
SD	4.62	3.88	0.39	6.74
t-Test: paired two sample of means				
t Stat	.042		1.107	
t Critical	4.303		4.303	

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