



ANALYSIS OF PARACETAMOL, PSEUDOEPHEDRINE AND BROMPHENIRAMINE IN COMTrex® TABLETS USING CHEMOMETRIC METHODS

Souha Hosam Youssef^{1*}, Maha Abdel Moneim Hegazy², Dalia Mohamed^{1,3},
Amr Mohamed Badawey⁴

¹Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, 11787, 6 October City, Egypt.

²Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt.

³Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, Ein Helwan, 11795, Cairo, Egypt.

⁴Pharmaceutical Chemistry Department, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt (FUE), 12311 Cairo, Egypt.

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*Corresponding Author

Souha Hosam Youssef

Pharmaceutical Analytical
Chemistry Department,
Faculty of Pharmacy,
October University for
Modern Sciences and Arts,
11787, 6 October City,
Egypt.

ABSTRACT

Paracetamol (PAR), pseudoephedrine hydrochloride (PSE) and brompheniramine maleate (BRM) are co-formulated drugs that are widely used in the Egyptian market for the relief of symptoms associated with common cold. Their severely overlapped spectra were resolved by two simple, accurate and precise chemometric techniques, principal component regression method (PCR) and partial Least Squares methods (PLS). The proposed methods were rapid, cost effective and were successfully applied for the analysis of laboratory prepared mixtures and the combined dosage form. Good recoveries were obtained for PCR method, 100.42, 100.05 and 98.96 % and for PLS method 100.04, 99.95 and 100.36 % for PAR, PSE and BRM, respectively. The methods were validated according to the ICH guidelines. Comparison of the applied methods with the reported

method was done and no significant difference was found regarding accuracy and precision.

KEYWORDS: Paracetamol; Pseudoephedrine; Brompheniramine; PLS; PCR.

1. INTRODUCTION

Modern life is moving at such a fast pace, consequently, a higher demand for effective and efficient over the counter medication is increasing everyday leading pharmaceutical companies to formulate more complex dosage forms. Therefore, a growing need for analyzing such mixtures was noted. Comtrex[®] Maximum Strength tablet is a ternary combination of paracetamol (PAR), pseudoephedrine hydrochloride (PSE) and brompheniramine maleate (BRM). It is widely used for relieving symptoms of colds, hay fever, and allergies such as headache, sinus pain, nasal and sinus congestion, sneezing, watery eyes, runny nose, fever, and itching of the nose or throat.

PAR; N-(4- hydroxyphenyl) acetamide is an analgesic and antipyretic used for treatment of pain caused by arthritis, tooth ache and headaches.^[1] It is a major ingredient in many cold and flu therapies. When PAR is combined with non-steroidal anti-inflammatory drugs or opioid analgesics; it can be used in controlling severe pain such as post-operative pain.^[2, 3] PAR is official in the British pharmacopoeia (BP).^[4] Literature survey revealed that PAR alone or in combination with other drugs was determined by titrimetry,^[4,5] spectrophotometry,^[6-12] spectrofluorimetry,^[13] thin layer chromatography (TLC),^[14-16] GC,^[17] HPLC-UV,^[18-23] HPLC-MS/MS^[24] and capillary electrophoresis (CE).^[25-27]

PSE; [(+)-threo-a-[1-methylamino) ethyl] benzyl alcohol] hydrochloride, is a sympathomimetic amine which directly acts on the adrenergic receptor system. It is often used for asthmatic patients due to its bronchodilator effect and treatment of nasal congestion by shrinking the swollen nasal mucous membranes.^[28] PSE is official in the BP.^[4] Several methods were found in the literature for its determination including titrimetry,^[4] spectrophotometry,^[29-32] TLC,^[15, 33-36] GC,^[37, 38] Micellar electrokinetic chromatography (MECK),^[39-41] HPLC-UV^[21-24, 30, 42-45] and capillary electrophoresis (CE).^[46-49]

BRM; (3RS)-3-(4-Bromophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine(Z)-butenedioate, is an antihistamine used for relieving allergy symptoms such as sneezing, itching and watery eyes.^[50] BRM is official in the BP.^[4] It has been recently released in the market; only two methods were found in the literature for BRM determination in combination with phenylephrine and in blood plasma, respectively.^[50, 51] In addition, the BP describes a titrimetric method for its determination.^[4] The structures of the three drugs are demonstrated in **Figure1**.

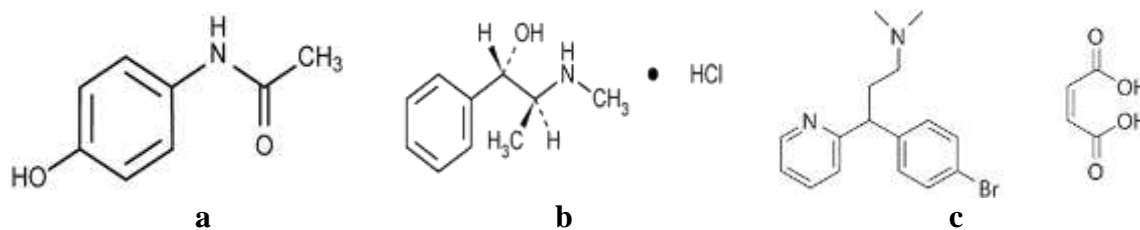


Fig. 1: Chemical structures of: a- paracetamol, b- pseudoephedrine and c- brompheniramine.

The quantitation of the proposed mixture was quite complicated due to the severe overlap of their spectra and the challenging dosage form ratio of 500: 30: 2 for PAR, PSE and BRM, respectively. As a result of the increase in the resolving power of analytical instrumentation and the easier access to microcomputers with appropriate software in recent years, the use of multivariate calibration data, that is, of the analytical signal depending on two or more variables, has become more general. Methods such as PCR and PLS have frequently been used in quantitative spectral analysis to obtain very selective information from unselective data.^[52]

Our aim was to conduct sensitive, accurate and precise chemometric methods for the determination of the three drugs in their combined dosage form specifically as to the extent of our knowledge; from a detailed literature survey that to date there is no reported method for their simultaneous determination.

2. MATERIALS AND METHODS

2.1. APPARATUS AND SOFTWARE

Shimadzu – UV 1800 double beam UV–Visible spectrophotometer (Japan) and 1 cm quartz cells at 200–400 nm range was used for measuring the absorbance. Spectral manipulations were carried out by Matlab for Windows™ version 7.9.

2.2. CHEMICALS AND SOLVENTS

Pure samples

PAR, PSE and BRM were kindly provided by GlaxoSmithKline (Cairo, Egypt). Their purities were found to be 99.40 ± 0.778 , 100.11 ± 0.427 and 99.12 ± 0.699 , respectively, according to the reported method of analysis.^[44]

Market sample

COMTRES[®] MAXIMUM STRENGTH coated tablets labeled to contain 500 mg of PAR, 30 mg PSE and 2 mg BRM (Batch number: A514875), manufactured by GlaxoSmithKline Egypt for Novartis Pharma Egypt, under license from Novartis Consumer Health, Switzerland and it was purchased from the local market.

Solvents

Double distilled water.

2.3. STANDARD SOLUTIONS

Stock solutions of concentrations 1000 µg/mL for PAR and BRM and 4000 µg/mL for PSE were separately prepared using distilled water as a solvent.

Working solutions were freshly prepared by dilutions from the stock solutions with distilled water as a solvent to obtain concentrations 100 µg/mL, 600µg/mL and 100 µg/mL for PAR, PSE and BRM, respectively.

2.4. PROCEDURE

Calibration and validation sets

A five level, three factor calibration design^[53] was applied using five concentration levels coded from +2 to -2 for each of the three components to be analyzed.

Twenty-five samples that constitute mixtures of PAR, PSE and BRM in different ratios were prepared by accurately transferring different aliquots from their working standard solutions into 10-mL volumetric flasks and the volumes were completed with distilled water. The final concentration ranges were 5.00-25.00 µg mL⁻¹, 100.00-180.00 µg mL⁻¹ and 5.00-25.00 µg mL⁻¹ for PAR, PSE and BRM, respectively.

Eight samples were randomly chosen and used as an external validation set and the rest of the samples were used for the construction of the regression model (calibration set).

Analysis of pharmaceutical dosage form (Comtrex[®] maximum strength tablets)

Ten Comtrex[®] maximum strength tablets were accurately weighted, grounded and mixed well. An equivalent amount to one tablet was accurately weighed and transferred into a beaker; the three components were extracted with 3 x 30 mL water. Then sonication was carried out for 15 minutes (for each extraction). The solution was filtered into a 100-mL

volumetric flask and completed to volume with water to obtain a solution (Stock 1) with the following concentrations $5000.0 \mu\text{g mL}^{-1}$ of PAR, $300.0 \mu\text{g mL}^{-1}$ of PSE and $20.0 \mu\text{g mL}^{-1}$ of BRM. An aliquot equivalent to 1 mL was accurately transferred from Stock 1 into a 100-mL volumetric flask and completed to volume with water to prepare a solution (Stock 2) with the concentration of $50 \mu\text{g mL}^{-1}$ of PAR, $3 \mu\text{g mL}^{-1}$ of PSE and $0.2 \mu\text{g mL}^{-1}$ of BRM. Finally, 4 mL from Stock 2 were accurately transferred to a 10-mL volumetric flask. The solution was then spiked with 2 mL PSE and 1 mL BRM from their corresponding working solutions and completed to volume with water forming a solution composed of 20, 121.2 and $10.08 \mu\text{g mL}^{-1}$ of PAR, PSE and BRM, respectively.

The absorption spectra of calibration set, validation set and pharmaceutical preparation were recorded in the range 220-300 nm at 1 nm intervals. The recorded spectra were then transferred to Matlab® 7.9 for subsequent data analysis.

3. RESULTS AND DISCUSSION

Chemometrics is an analysis method that uses mathematics, statistics and formal logic to design or select optimal experimental procedures, to provide maximum relevant information by analyzing chemical data and to obtain knowledge about chemical systems. The reason for the emergence of chemometrics was twofold, introduction of instrumentation giving multivariate responses for each sample analyzed and the availability of computers and growing wealth of good software for such purposes.^[54]

The absorption spectra of PAR, PSE and BRM are shown in Figure (2). It is observed from the figure that the three spectra show severe overlapping. This overlap could be resolved using multivariate calibration methods as PCR and PLS. So, these methods were applied for the determination of the three drugs in their laboratory prepared mixtures as well as in pharmaceutical preparation.

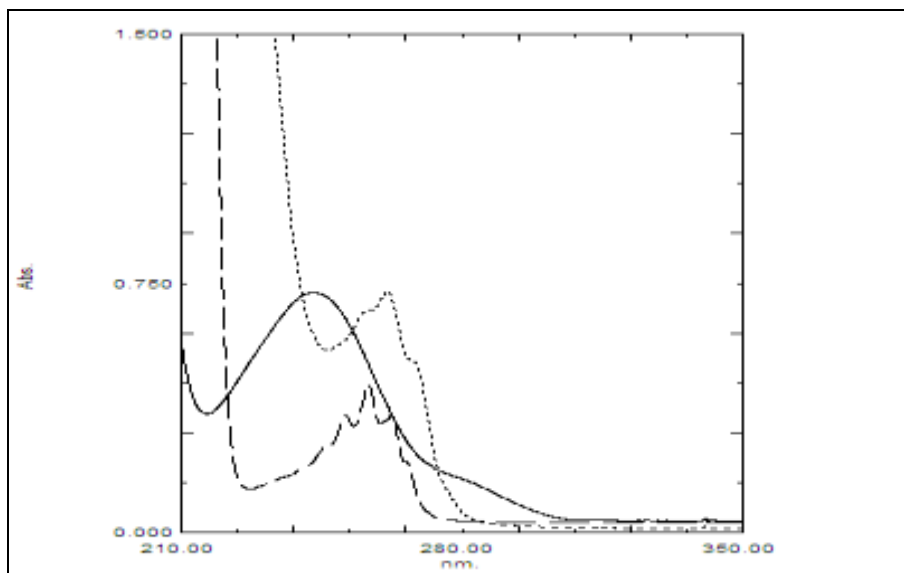


Fig. 2: Zero – order absorption spectra of 10.00, 600.00 and 50.00 $\mu\text{g mL}^{-1}$ of PAR (—), PSE (- - -) and BRM (.....), respectively.

The prepared samples were scanned in the range of 220-300 nm with 1 nm intervals, thus producing 81 data points per spectrum. The produced spectral data matrix has 25 rows representing different samples and 81 columns representing wavelengths (25 x 81). Seventeen samples were randomly chosen and used for calibration and eight were used as an external validation set. The concentrations of PAR, PSE and BRM in each mixture are shown in Table (1).

Table (1): Concentrations of PAR, PSE and BRM in the calibration and validation set for PLS and PCR.

Mixture	Paracetamol ($\mu\text{g mL}^{-1}$)	Pseudoephedrine ($\mu\text{g mL}^{-1}$)	Brompheniramine ($\mu\text{g mL}^{-1}$)
1	15	140	15
2	15	100	5
3	5	100	25
4	20	100	15
5	25	120	25
6	10	180	15
7	10	140	5
8	15	120	10
9	10	120	20
10	10	100	10
11	20	180	20
12	25	160	15
13	20	140	25
14	15	180	25

15	25	180	5
16	25	100	20
17	5	160	5
18	5	180	10
19	5	140	20
20	15	160	20
21	20	160	10
22	20	120	5
23	10	160	25
24	5	120	15
25	25	140	10

*Mixtures 4,7,10, 17, 19, 20, 21 and 22 are those of the validation set.

In order to apply PCR^[55, 56] and PLS^[57] methods to the data, the raw data of the calibration samples were mean centered as a processing step and random subsets were applied as an internal cross validation method. To choose the optimum number of latent variables (LV), F statistics were used in which the root mean squares error of cross validation (RMSECV) for each model were computed. After constructing the PCR and PLS models, it was found that the optimum number of LVs described by the developed models was three in PCR and PLS, as shown in Figures (3 and 4).

The RMSECV was calculated for each method as follows.

$$\text{RMSECV} = \sqrt{(\text{press}/n)}$$

Where press is the predicted residual error sum of squares, n is the number of calibration set samples, and is calculated as follows:

$$\text{Press} = \sum (Y_{\text{pred}} - Y_{\text{true}})^2$$

Where Y_{pred} and Y_{true} are predicted and true concentrations in $\mu\text{g mL}^{-1}$, respectively.

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations.

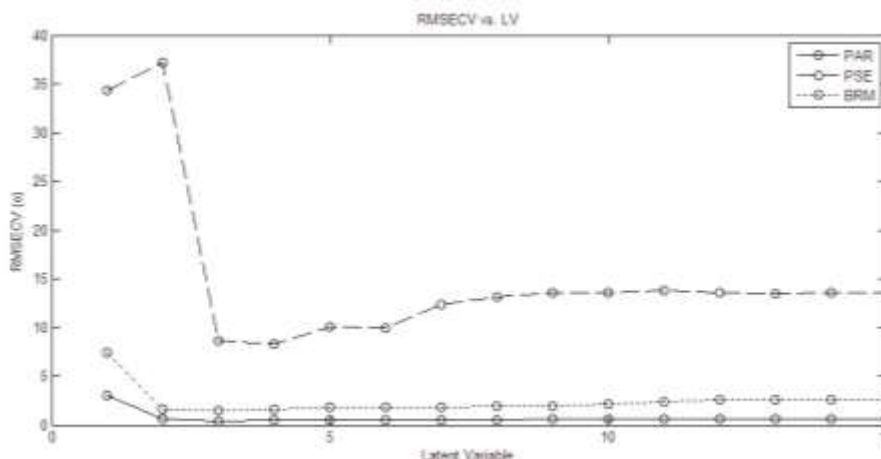


Fig. 3: Cross validation results of the training set as a function of the number of latent variables used to construct the PLS calibration models.

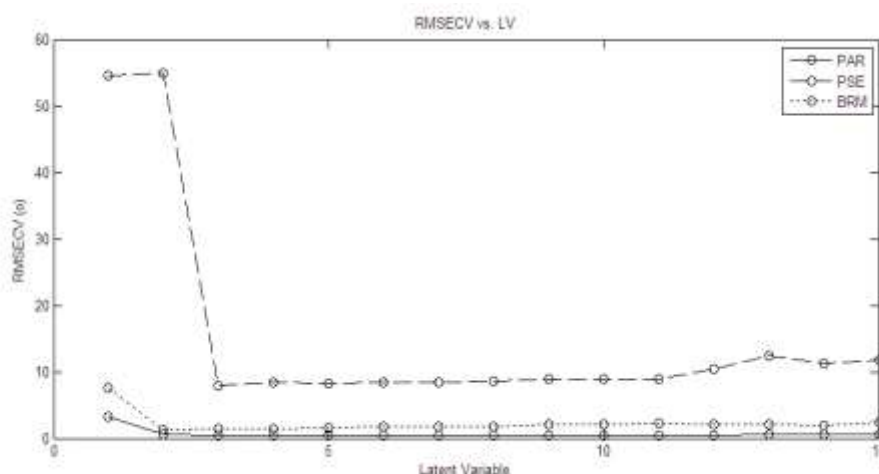


Fig. 4: Cross validation results of the training set as a function of the number of latent variables used to construct the PCR calibration models.

For testing the validity of the developed models, an external validation set was used. The recoveries, mean concentrations, relative standard deviation and root mean squares error of prediction (RMSEP) values are summarized in Table (2), PLS models show better prediction relation to PCR models as indicated by the smaller RMSEP. The RMSEP was used as a diagnostic tool for examining the prediction of the developed models^[58]; it has indicated both accuracy and precision. The regression equations parameters of the linear relationship between the calculated and the true concentrations of PAR, PSE and BRM in the validation set are represented in Table (3).

Table (2): Percentage recoveries of PAR, PSE and BRM in the validation set using PCR and PLS models.

Sample no.	Concentration ($\mu\text{g mL}^{-1}$)			Recovery %					
				PCR			PLS		
	PAR	PSE	BRM	PAR	PSE	BRM	PAR	PSE	BRM
1	5	160	5	100.20	101.07	99.32	100.34	99.91	101.76
2	20	100	15	100.02	100.04	97.65	100.10	99.84	100.67
3	5	140	20	100.20	100.58	97.74	98.68	100.19	99.29
4	20	160	10	99.37	99.06	101.63	99.87	100.13	98.70
5	20	120	5	99.47	101.02	98.96	100.23	99.68	101.74
6	10	100	10	100.66	99.62	99.24	98.74	100.06	99.43
7	5	120	15	100.66	98.95	99.32	101.69	99.76	101.66
8	10	140	5	102.76	100.06	97.82	100.65	100.02	99.64
Mean				100.42	100.05	98.96	100.04	99.95	100.36
\pm SD				1.059	0.814	1.308	0.985	0.181	1.249
RMSEP				0.118	0.969	0.248	0.0662	0.222	0.127

Table (3): Regression and parameters for model validation of the proposed chemometric methods.

Validation parameter	PAR		PSE		BRM	
	PCR	PLS	PCR	PLS	PCR	PLS
Predicted vs. known conc. Plot						
Slope	0.991	1.00	0.991	0.999	0.974	0.997
SE slope	6.81×10^{-3}	4.10×10^{-3}	1.73×10^{-2}	1.11×10^{-2}	9.91×10^{-3}	9.60×10^{-3}
Intercept	0.142	-0.00487	1.20	0.112	0.172	0.0494
Correlation coefficient (r)	0.9997	0.9999	0.9991	0.9996	0.9994	0.9997
SE of regression	0.0985	0.0556	2.28	1.46	0.136	0.114

It is clear from the obtained results that there is no significant difference between the PCR and PLS models, all models were successfully applied for the determination of PAR, PSE and BRM in Comtrex® maximum strength tablets with good recoveries. The accuracy of the models was further assessed by applying the standard addition technique as shown in Table (4).

Table (4): Analysis of PAR, PSE and BRM in Comtrex[®] maximum strength tablets and application of standard addition technique using the proposed PCR and PLS methods.

Drug	Chemometric methods			
	Claimed amount taken	Added	Recovery% ^a	
PCR			PLS	
PAR	20.0 ($\mu\text{g mL}^{-1}$) [100.37 ^b]	1.5	96.19	97.65
		2.0	97.78	97.33
		2.5	96.04	98.89
	Mean \pm SD		96.67 \pm 0.964	97.96 \pm 0.824
PSE	121.2 ($\mu\text{g mL}^{-1}$) [97.87 ^b]	25.0	97.21	96.52
		35.0	95.41	95.59
		50.0	95.98	97.15
	Mean \pm SD		96.20 \pm 0.920	96.42 \pm 0.785
BRM	10.08 ($\mu\text{g mL}^{-1}$) [97.52 ^b]	5.0	96.25	97.58
		10.0	95.81	96.69
		12.5	97	98.02
	Mean \pm SD		96.35 \pm 0.602	97.43 \pm 0.678

The results obtained by the proposed methods were statistically compared to those obtained by applying the reported method^[44, 50] and no significant difference were observed between the PCR and PLS methods and the reported one with respect to accuracy and precision, Table (5).

Table (5): Statistical comparison of the results obtained by the proposed PCR and PLS methods and reference methods for the determination of PAR, PSE and BRM.

	PAR		Reference ^[44]	PSE		Reference ^[44]	BRM		Reference ^[50]
	PCR	PLS		PCR	PLS		PCR	PLS	
Mean	100.42	100.04	99.40	100.05	99.89	100.11	98.96	100.36	99.12
SD	1.06	0.985	0.778	0.814	0.186	0.427	1.31	1.25	0.699
N	8	8	4	8	5	4	8	8	4
Variance	1.12	0.970	0.605	0.663	0.0346	0.182	1.716	1.563	0.489
Student's t	1.70 (2.23)	1.25 (2.23)		0.13 (2.23)	1.06 (2.36)		0.232 (2.23)	1.81 (2.23)	
F	1.85 (8.89)	1.60 (8.89)		3.64 (8.89)	5.26 (6.59)		3.51 (8.89)	3.20 (8.89)	

Figures between parentheses represent the corresponding tabulated values of *t* and *F* at *P* = 0.05.

The reported method for the determination of PAR and PSE is an HPLC method using C18 column, a mobile phase composed of 25mM phosphate buffer (pH=5): methanol: acetonitrile (30:60:10, v/v/v) at flow rate 1mL/min and detection at 240 nm.

The reported method for the determination of BRM is a TLC using methanol: ammonia (100:1.5 v/v) as mobile phase.

4. CONCLUSION

The proposed chemometric methods are simple, accurate and selective for the determination of PAR, PSE and BRM without preliminary separation in pure form or in their pharmaceutical forms. The methods have shown a number of advantages where fewer manipulations was required compared to other spectrophotometric methods and high speed at which the components were determined in mixtures. Moreover, the suggested methods were inexpensive and environment friendly since only water was used as a solvent.

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