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Simultaneous determination of pipenzolate bromide and phenobarbitone in pharmaceutical preparations by HPLC method

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ABSTRACT

A binary mixture of pipenzlate bromide and phenobarbitone was determined by HPLC method using 0.05 M ammonium dihydrogen phosphate/acetonitrile/methanol (7:12:1, by volume) as the mobile phase with UV detection at 220 nm over concentration ranges of 10-90 μg. ml^{-1} and 1-80 μg. ml^{-1} with mean percentage accuracies 99.90 ± 0.62 and 100.30 ± 0.92 for pipenzlate bromide and phenobarbitone, respectively. The suggested procedure was checked using laboratory prepared mixtures and was successfully applied for the analysis of their pharmaceutical preparation. The method retained its accuracy and precision when applying the standard addition technique. The results obtained by applying the proposed method were statistically analyzed and compared with those obtained by the manufacturer method.

KEYWORDS

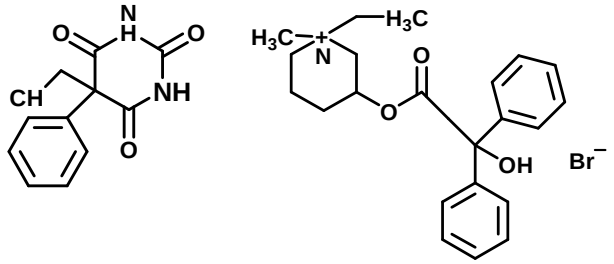
Pipenzlate bromide;
Phenobarbitone;
Reversed phase HPLC.

INTRODUCTION

Pipenzolate bromide, 1-Ethyl-3-[(hydroxydiphenylacetyl) oxy]-1-methylpiperidinium bromide[1], Figure 1. It is quaternary ammonium antimuscarinic agent with peripheral actions similar to those of atropine. It is used as adjunct in the treatment of gastrointestinal disorders characterized by smooth muscle spasm[2]. The literature survey reveals few methods for determination of pipenzolate bromide alone[3,4], in combined mixture with phenobarbitone[5,6] or chlordiazepoxide[7,8] including HPLC[3], capillary electrophoresis[4], colorimetric methods[5,6] and spectrophotometric methods[7,8].

Phenobarbitone, 5-ethyl-5-phenyl-2,4,6(1H,3H, 5H)-pyrimidinetrione, Figure 1, is a hypnotic drug used as sedative and anticonvulsant agent[1].

Several methods have been reported for the determination of phenobarbitone alone, in biological fluids and in pharmaceutical preparations including HPLC[9-13], spectrophotometric method[13], chemometric method[12], thin layer chromatography[14], gas chromatography with mass spectrometry[15] and capillary electrophoresis[16,17].



Pipenzolate bromide $C_{22}H_{28}BrNO_3$

Phenobarbitone $C_{12}H_{12}N_2O_3$

Figure 1 : Chemical structure of pipenzolate bromide and phenobarbitone

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Pipenzolate bromide is present in combination with phenobarbitone in the drug formulation named "Babytal drops". Phenobarbitone is added to potentate the spasmolytic action of pipenzolate bromide, to sedate the child and to relive anxiety which usually associates colics.

Only two colorimetric methods[5,6] are available for determination of pipenzolate bromide in presence of low concentration of phenobarbitone. No method is available for simultaneous determination of the two drugs. So there was a need to develop simple and accurate method for their determination in combination. The purpose of this study was to determine both drugs concurrently by simple, rapid and selective HPLC assay for quality control and routine analysis.

EXPERIMENTAL

Apparatus

The chromatography was performed on a Agilent instrument, Model Agilent 1100 series U.S.A., equipped with a variable- wavelength detector and a 20-µl volume injection loop. Zorbax ODS (5 µm, 25 cm x 4.6 mm I.D.) column was used as stationary phase.

Materials

Pure samples

Pipenzolate bromide and phenobarbitone, working standards, were kindly supplied by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt. Their purity was found to be 100.88 ± 0.44 % and 100.34 ± 0.76%, respectively, according to manufacturer's method[18].

Market samples

Babytal drops (Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt). Each one ml contains 4 mg of Pipenzolate bromide and 6 mg of phenobarbitone.

Chemicals and reagents

Ammonium dihydrogen phosphate (E- Merck, Darmstadt, Germany) was of pure analytical grade, deionized water, methanol and acetonitrile (E- Merck, Darmstadt, Germany) were of HPLC grade.

Standard solutions

Stock solutions

Pipenzolate bromide (I) and phenobarbitone (II) stock solution (1.0 mg. ml^{-1}) each were prepared by weighing accurately 100 mg of (I) and 100 mg of (II) powder into two separate 100-ml measuring flasks. 50 ml of methanol was added, shacked for few minutes and completed to volume with the same solvent.

Working solutions

5 ml of the stock solutions of (I) and (II) were transferred in two separate 50-ml measuring flasks and diluted to the mark with methanol to get a final concentration of (100 µg. ml^{-1}) of (I) and (II).

Laboratory-prepared mixtures

Aliquot 3, 4, 5, 6, 4, 4 ml of (I) from its working solution (100 µg. ml^{-1}) were transferred into a series of 10-ml measuring flasks and aliquot 6, 6, 6, 6, 5, 7 ml of (II) using its working solution (100 µg. ml^{-1}) were added to the same flasks, completed to volume with methanol and mixed well.

Procedures

Linearity

Accurate aliquot equivalent to 100-900 µg of (I) from its working solution (100 µg. ml^{-1}) and aliquot equivalent to 10-800 µg of (II) from its working solution (100 µg. ml^{-1}) were transferred into two separate set of 10-ml measuring flask and completed to volume with methanol. Using an Agilent instrument, the chromatogram was recorded under the following instrumental parameters: flow rate was 1 ml min^{-1} at ambient temperature and the elluent was monitored at 220 nm. The separation was done on a C18 column 0.05 M ammonium dihydrogen phosphate: acetonitrile: methanol (7:12:1, by volume) as a mobile phase. Calibration curves for both pipenzolate bromide and phenobarbitone were plotted and the corresponding regression equations were calculated.

Assay of laboratory-prepared mixtures

The chromatographic conditions were applied for each laboratory-prepared mixture and the concentrations of pipenzolate bromide and phenobarbitone in these mixtures were calculated by substituting in the regression equations.

Application to pharmaceutical preparation (Babytal drops)

Accurate aliquot of 1 ml from the mixed con-

tents of 2 Babytal drops, equivalent to 4 mg pipenzolate bromide and 6 mg phenobarbitone, was transferred accurately to a 100-ml measuring flask and completed to the volume with methanol and analyzed according to instrumental parameters as under linearity.

RESULTS AND DISCUSSION

New methods for simultaneous determination of two or more compounds in the same sample, without previous chemical separation, are always of interest.

This work is devoted for the simultaneous determination of pipenzolate bromide and phenobarbitone which are available together in the form of drops. However, by reviewing the literature concerned the determination of (I) and (II) in mixture, it was found that no literature was reported for simultaneous determination of the two drugs. Therefore, the aim of this work was to develop simple analytical method for the simultaneous determination of two drugs. This was achieved using HPLC method.

To optimize the proposed HPLC method, all of the experimental conditions were investigated. For the choice of the stationary phase, reversed-phase separation was preferred to normal phase due to the draw backs of the normal phase, e.g.: hydration of silica with water which can cause peak tailing.

Concerning the mobile phase, different systems were tried for chromatographic separation of the two components by combining homogenous design and solvent polarity optimization. The best resolution was achieved when using a mobile phase consisting of 0.05 M ammonium dihydrogen phosphate:acetonitrile:methanol (7:12:1, by volume) which gave a better resolution and sensitivity of both drugs, Figure 2.

System suitability tests of HPLC method show good resolution for the two drugs, TABLE 1

Linear relation was obtained between peak area and the concentration of the two drugs in the range of (10-90 μg. ml^{-1} and 1-80 μg. ml^{-1}) for pipenzolate bromide and phenobarbitone, respectively.

The linear regression equations were computed and found to be:

Y = 2.0256X – 0.1594 r = 0.9999 (for pipenzolate bromide)

Y = 2.813X + 21.096 r = 0.9999 (for phenobarbitone)

Where Y is the area under the peak, X is the concentration in μg. ml^{-1} and r is the correlation coefficient.

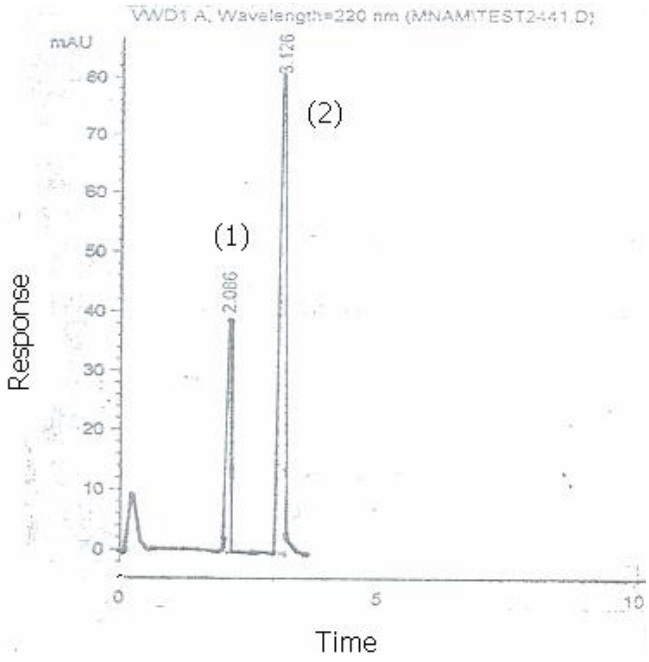

Figure 2 : HPLC chromatogram of 40 μg.ml^{-1} pipenzolate bromide (1) and 60 μg.ml^{-1} phenobarbitone (2).

TABLE 1 : Statistical analysis of the parameters required for system suitability test of HPLC method.

Parameter	Obtained value		Reference value
	Pipenzolate bromide	Phenobarbitone	
Resolution (R)	3.47		R > 0.8
T (Tailing factor)	1.25	1	T= 1 for a typical symmetric peak
α (relative retention time)	1.56		> 1
K (column capacity)	4.53	8.86	1-10 acceptable
N (column efficiency)	1741.56	971.19	Increases with efficiency of the separation
HETP	0.0143	0.0257	The smaller the value, the higher the column efficiency

Results obtained by applying HPLC procedure showed that pipenzolate bromide and phenobarbitone can be simultaneously analyzed in the prepared mixtures with mean percentage recoveries of 99.40 ± 0.45 and 100.40 ± 0.39, respectively, TABLE 2.

The proposed method has been applied to assay

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TABLE 2 : Determination of pipenzolate bromide and phenobarbitone in laboratory–prepared mixtures by HPLC method.

Mixture NO.	Pipenzolate bromide			Phenobarbitone		
	Claimed taken $\mu g.ml^{-1}$	Found* $\mu g.\ ml^{-1}$	Recovery %	Claimed taken $\mu g.ml^{-1}$	Found* $\mu g.\ ml^{-1}$	Recovery %
1	30	29.763	99.21	60	60.348	100.58
2	40	39.788	99.47	60	60.594	100.99
3	50	50.115	100.23	60	60.294	100.49
4	60	59.394	98.99	60	60.108	100.18
5	40	39.772	99.43	50	50.180	100.36
6	40	39.628	99.07	70	70.056	100.08
Mean ± S.D.%			99.40 ± 0.45			100.40 ± 0.39

*** Average of four determinations.**

pipenzolate bromide and phenobarbitone in Babytal drops. The validity of the suggested procedures was further assessed by applying the standard addition technique, TABLE 3.

TABLE 3 : Application of standard addition technique to the analysis of pipenzolate bromide and phenobarbitone in babytal drops by proposed HPLC method.

Babytal drops (batch no. 0460310)	Found* %	Pure added $\mu g.\ ml^{-1}$	Pure found* $\mu g.\ ml^{-1}$	Recovery %
Pipenzolate bromide	99.57 ± 0.49	10	9.972	99.72
		20	20.032	100.16
		30	29.823	99.41
		40	40.152	100.38
Mean ± S.D. %				99.92±0.44
Phenobarbitone	100.24 ± 0.39	10	10.064	100.64
		20	20.012	100.06
		30	30.081	100.27
		40	39.916	99.79
Mean ± S.D. %				100.19±0.36

***Average of four determinations.**

A statistical comparison of the results obtained by the proposed method and the manufacturer procedure that depends on colorimetric determination of pipenzolate bromide and U.V. spectrophotometric determination of phenobarbitone[18] for pure drugs is shown in TABLE 4. The values of the calculated t and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed method and the manufacturer procedure.

The results of assay validation of the proposed method show that the methods are accurate, precise, specific and rugged according to RSD% of intraday and interday determinations, TABLE 5.

TABLE 4 : Statistical analysis of the results obtained by the proposed HPLC method compared with manufacturer method[18] for the analysis of pipenzolate bromide and phenobarbitone.

Values	The proposed HPLC method		Manufacturer method[18]	
	Pipenzolate bromide	Phenobarbitone	Pipenzolate bromide	Phenobarbitone
Mean ± S.D. %	100.48 ± 0.54	100.30 ± 0.92	100.88 ± 0.44	100.34 ± 0.76
N	6	6	5	5
Variance	0.292	0.846	0.194	0.578
t (2.262)*	1.326	0.078	----------	----------
F (5.19)*	1.503	1.464	----------	----------

*** The values in parenthesis are corresponding to the theoretical values of t and F at (p = 0.05).**

TABLE 5 : Validation of the results obtained by applying the proposed HPLC method for the analysis of pipenzolate bromide and phenobarbitone.

Parameters	Pipenzolate bromide	Phenobarbitone
Range	10-90 $\mu g.\ ml^{-1}$	1-80 $\mu g.\ ml^{-1}$
Slope	2.0256	2.813
Intercept	-0.1594	21.096
Mean ± S.D. %	100.48 ± 0.54	100.30 ± 0.92
Correlation Coefficient (r)	0.9999	0.9999
RSD%[a]*	0.83 – 0.54	0.55 – 0.98
RSD%[b]*	0.39 – 0.71	0.48 – 0.73

***[a,b] Intraday and interday (n=4) relative standard deviations of samples of concentrations (20,50 $\mu g.\ ml^{-1}$) of pipenzolate bromide and phenobarbitone, respectively.**

CONCLUSION

The proposed procedure suggested that, it could be applied for the simultaneous determination of pipenzolate bromide and phenobarbitone. Moreover, the method is rapid, sensitive, selective and could be used in routine and quality control analysis.

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