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Simultaneous determination of paracetamol and methocarbamol in tablets by ratio spectra derivative spectrophotometry and LC

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Abstract

The application of the ratio spectra derivative spectrophotometry and high-performance liquid chromatography (HPLC) to the simultaneous determination of paracetamol (PAR) and methocarbamol (MET) in combined pharmaceutical tablets is presented. The spectrophotometric procedure is based on the use of the first derivative of the ratio spectra obtained by dividing the absorbtion spectrum of the binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes were measured at 243.0 and 230.3 nm for the assay of PAR and MET, respectively. Calibration graphs were established for 2–30 μg/ml for PAR and 2–36 μg/ml for MET in binary mixture.The detection limits for PAR and MET were found 0.097 and 0.079 μg/ml, respectively; while the quantification limits were 0.573 μg/ml for PAR and 1.717 μg/ml for MET. For the HPLC procedure, a reversed-phase column with a mobile phase of methanol-water (60:40, v/v), was used to separate both compounds with a detection of 274.0 nm. Linearity was obtained in the concentration range of 2–300 and 1.5–375 μg/ml for PAR and MET, respectively. The detection and quantification limits were found to be 0.42 and 1.4 μg/ml for PAR and 0.36 and 1.2 μg/ml for MET, respectively. The relative standard deviations were found to be less than 0.52%, indicating reasonable repeatibility of both methods. The proposed methods were successfully applied to the determination of these drugs in commercial tablets.

Keywords: Bulk and tablets; High-performance liquid chromatography; Methocarbamol; Paracetamol; Ratio spectra derivative spectrophotometry

1. Introduction

Paracetamol (PAR) and methocarbamol (MET) are used in association for myorelaxan and analgesic purposes. A literature survey reveals very

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few analytical reports for the analysis of both drugs in combined dosage forms based on spectrophotometry [1], derivative spectrophotometry [2], gas liquid chromatography [3,4] and high-performance liquid chromatography [5]. The PAR–MET mixture is not yet official in any pharmacopoeia.

The classic analytical problem of spectrophotometric multicomponent analysis is that the analyte of interest is often accompanied by other compounds absorbing in the same spectral region. In these cases, spectral overlapping requires resolution by mathematical procedures. Recently, Salinas et al. [6] developed a new spectrophotometric method, named the ratio derivative spectrophotometry for resolving binary mixtures. This method has been succesfully applied to spectroscopic data [7–10].

The present paper describes ratio spectra derivative spectrophotometry for the simultaneous determination of PAR and MET in bulk material and in pharmaceutical tablets.

As an alternative method, reversed-phase high performance liquid chromatography was also used for the determination of both drugs in the presence of each other.

2. Experimental

2.1. Apparatus

Spectrophotometric studies were carried out with a Shimadzu 1601 double beam UV-Vis spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software, and equipped with a Lexmark printer.

High-performance liquid chromatography (HPLC) analysis was performed on a Waters chromatograph (Model 510) equipped with a UV detector (Model 481). The chromatograms were analysed with a chromatographic workstation (Baseline 810).

2.2. Materials

Paracetamol (PAR) and methocarbamol (MET) were kindly supplied by Sanofi-Doğu Pharm. Ind., Turkey.

Methanol was HPLC grade; water was doubly distilled from all glass apparatus. Stock solutions of 1 mg/ml of the drugs were prepared in methanol. Standard solutions for the spectrophotometric measurements were prepared by serial dilution to contain the concentration required for the calibration curves.

Standard solutions for HPLC were prepared with the mobile phase by varying the concentration of one of the drugs and maintaining the other one at a constant level of 10 μg/ml. The samples were filtered before injection into the chromatograph.

2.3. Analysis of tablets

A total of 20 tablets containing PAR and MET as active ingredients were weighed and finely powdered. An amount corresponding to one tablet was accurately weighed, transferred to a 100-ml volumetric flask, stirred with methanol, made up to volume with the same solvent and filtered. Further dilution was made using either methanol (for ratio spectra derivative procedure) or mobile phase (for HPLC procedure).

2.4. Procedures

2.4.1. Ratio spectra derivative spectrophotometry

Standard solutions of PAR and MET containing concentration ranges of 2–30 and 2–36 μg/ml were prepared in methanol, respectively.

The absorbtion spectra of the binary mixtures prepared at different concentrations of PAR were recorded and stored in the IBM PC computer. The stored spectra of the mixtures were divided by a standard spectrum of MET solution (10 μg/ml in methanol). From the ratio spectra thus obtained, first derivatives calculated with $\Delta\lambda = 4$ nm, were recorded. In the binary mixtures we can determine the amount of PAR by measuring the first derivative signals at 243.0 nm in the range of 231.6–270.8 nm.

The similar procedure was followed for the different concentrations of MET when PAR was 12.0 μg/ml. In the same way as described above, the content of MET was determined by measuring the signals in the first derivative of ratio spectrum at 230.3 nm in the range of 200.8–245.5 nm.

2.4.2. High-performance liquid chromatography

Chromatographic separation was performed on a reversed-phase Supelcosil LC-18 (250 × 4.6 mm, 5 μm particle size) column. The compounds were separated with a mobile phase consisting of a mixture of methanol-water (60:40, v/v). The mobile phase was filtered and sonicated before use, and delivered at a flow rate of 1.0 ml/min. (The injection volume was 50 μl). Calibration curves were constructed by plotting the peak area against the relative concentrations of each drug. Standard solutions were prepared with mobile phase by varying concentration of PAR and MET in the range of 2–300 and 1.5–375 μg/ml, respectively.

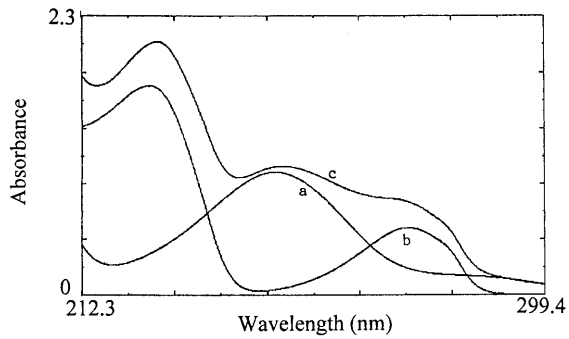

Fig. 1. Zero-order spectra of (a) 25 μg/ml PAR; (b) 10 μg/ml MET; and (c) their mixture in methanol.

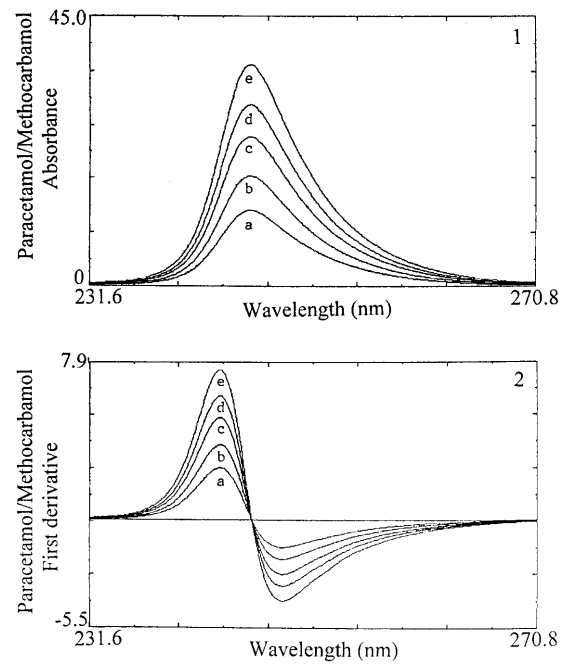

Fig. 2. Ratio spectra (1); and first derivatives (2) of PAR of (a) 2 μg/ml; (b) 7 μg/ml; (c) 12 μg/ml; (d) 21 μg/ml; and (e) 30 μg/ml, using a 10 μg/ml MET as divisor.

3. Results and discussion

3.1. Ratio spectra derivative spectrophotometric method

Fig. 1 shows the absorption spectra corresponding to PAR, MET and a mixture of them in methanol. Both components with sufficiently overlapped spectra in binary mixture can be evaluated by the proposed ratio derivative spectrophotometric method.

Fig. 2 shows the ratio spectra of different amounts of PAR (spectra divided by the standard spectrum of a 10 μg/ml solution of MET) and their first derivatives. The first derivative amplitudes at 243.0 nm corresponding to a maximum wavelength are proportional to the PAR concentration.

For determining the other component, MET, an analogous procedure was followed. Fig. 3 shows the ratio spectra of different standard solutions of MET and their first derivatives, using the spectrum of a 12 μg/ml solution of PAR as the divisor. The concentration of MET is proportional to the amplitude of the minimum at 230.3 nm.

Statistical analysis of the regression equations is reported in Table 1.

The ratio spectra derivative method permits the use of the different concentrations as the divisor to obtain the different calibration graphs. For selecting the standard solution as divisor at an appropriate concentration, concentrations of both drugs in the range 2–30 μg/ml were tested. A concentration of 10 μg/ml of MET and 12 μg/ml of PAR as divisor gave highest correlation coefficient values, being an indication of the quality of the fitting of the data to the straight line. The influence of $\Delta\lambda$ for the first derivative spectra was also tested. It was found to be suitable to use the value of $\Delta\lambda = 4$ nm.

Table 1
Statistical analysis for the calibration graphs of PAR and MET in mixtures by use of the first derivative ratio spectra and HPLC methods

Method	Analyte	Wavelength (nm)	Linearity range (μg/ml)	Regression equation[a]	Correlation coefficient	RSD of slope	RSD of intercept	Detection limit (μg/ml)	Quantification limit (μg/ml)
Ratio spectra	PAR	243.0	2–30	$Y = 2.75 \times 10^{-3}C + 5.98 \times 10^{-4}$	0.9987	0.081	0.21	0.097	0.573
derivative	MET	230.3	2–36	$Y = 2.83 \times 10^{-3}C + 8.78 \times 10^{-4}$	0.9995	0.099	0.12	0.079	1.72
spectrophotometry	PAR	274.0	2–300	$Y = 0.179C + 0.375$	0.9998	0.32	0.11	0.42	1.4
HPLC	MET	274.0	1.5–375	$Y = 0.0374C + 0.0423$	0.9999	0.55	0.47	0.36	1.2

[a] Where C is the concentration of the analyte (μg/ml).

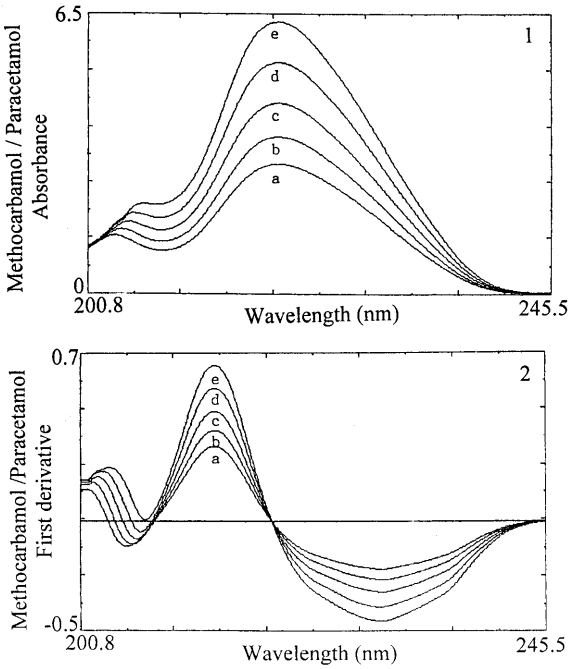

Fig. 3. Ratio spectra (1); and first derivatives (2) of MET of (a) 2 μg/ml; (b) 8 μg/ml; (c) 10 μg/ml; (d) 23 μg/ml; and (e) 36 μg/ml, using a 12 μg/ml PAR as divisor.

In this work, nine synthetic mixtures of PAR and MET were analysed by the proposed method. The results are given in Table 2.

3.2. HPLC method

The reversed phase HPLC method was developed as referee method for the ratio spectra derivative assay. In order to use the similar sample that in the spectrophotometric analysis, the mobile phase was chosen as methanol-water (60:40 v/v) after several trials in various proportions. According to USP24, method ⟨621⟩, system suitability tests are an integral part of a chromatographic method. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solutions of PAR and MET. Resolution and selectivity factors for this system were found 3.69 and 2.97, respectively. Tailing factors and capacity factors were obtained as 1.08 and 1.03 for PAR and 1.06 and 3.05 for MET, respectively.

As shown in Fig. 4, the retention times were 3.65 min for PAR and 7.3 min for MET. The linearity of the detector response for both drugs was determined by plotting peak area ratios *vs* concentration.The detection limit was calculated as the intercept of the calibration graphs plus three times the estimated standard deviation. The analytical data for the calibration graphs are listed in Table 1.

In order to demonstrate the validity and suitability of the proposed method, intra- and inter-day variability studies were performed for two different concentrations (Table 3). These results

Table 2
Resolution of PAR and MET laboratory-made mixtures by using the first derivative ratio spectra

Taken (mg/100 ml)		Found (mg/100 ml)		Recovery (%)	
Paracetamol	Methocarbamol	Paracetamol	Methocarbamol	Paracetamol	Methocarbamol
300.0	325.0	299.4	324.3	99.8	99.8
300.0	350.0	297.0	347.8	99.0	99.4
300.0	375.0	297.0	374.4	99.0	99.8
300.0	400.0	296.5	395.2	98.8	98.8
300.0	425.0	297.8	424.3	99.3	99.8
200.0	375.0	198.7	373.0	99.3	99.5
250.0	375.0	245.1	373.0	98.0	99.5
350.0	375.0	347.9	373.1	99.4	99.5
400.0	375.0	398.0	373.6	99.5	99.6
				Mean: 99.1	Mean: 99.5
				RSD%: 0.52	RSD%: 0.31

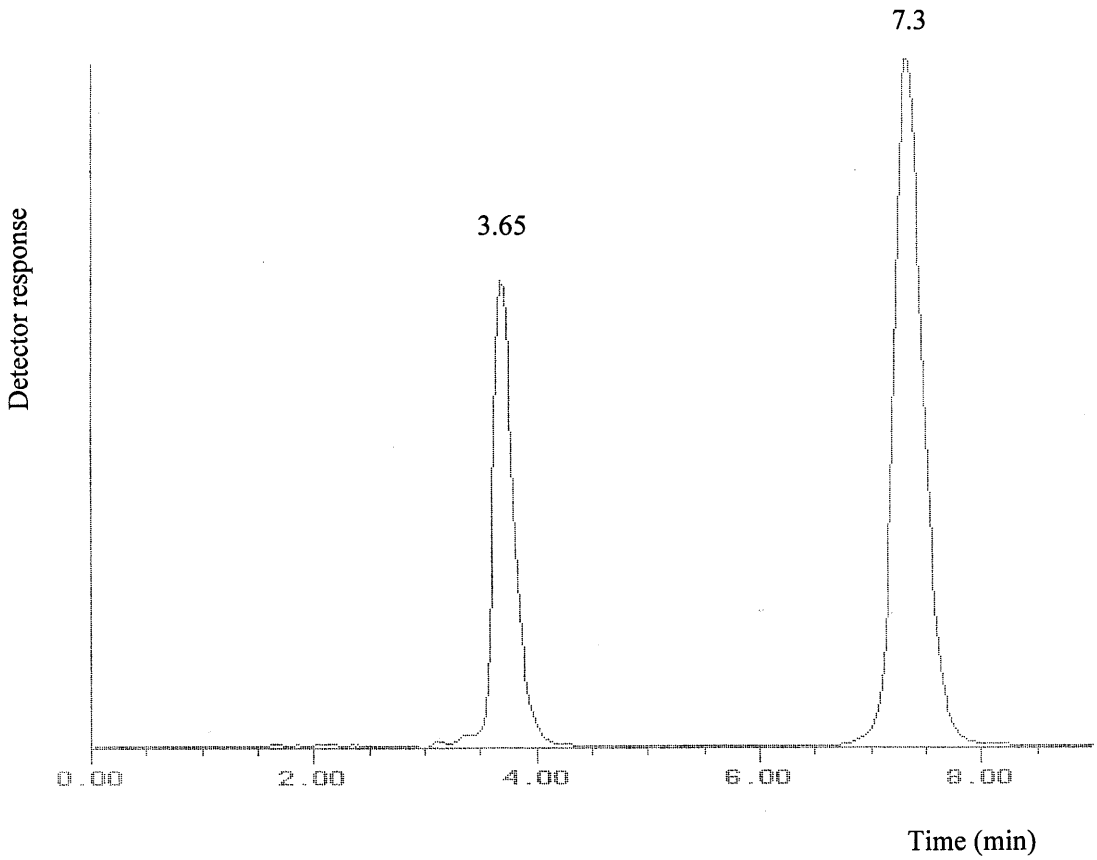

Fig. 4. Chromatogram of a solution of PAR (1) (10 μg/ml); and MET (2) (50 μg/ml) in the mobile phase.

Table 3
Intraday and interday precision of PAR and MET standards by using HPLC method

Sample	Theoretical concentration (μg/ml)	Intra day measured concentration[a]		Inter day measured concentration[b]	
		Mean (μg/ml)	RSD%	Mean (μg/ml)	RSD%
Paracetamol	50	49.97	0.35	49.64	0.46
	100	99.77	0.18	99.68	0.23
Methocarbamol	50	49.93	0.40	49.63	0.34
	100	99.91	0.20	99.59	0.32

[a] Mean value of five different standards for each concentration.
[b] Inter day reproducibility was determined from five different runs over a 2 weeks period.

show the accuracy, reproducibility and repeatability of the assay. Thus, it was concluded that, there was no significant difference for the assay which was tested within day and between day.

Recovery test also confirmed the accuracy and applicability of the proposed method by analyzing synthetic mixtures of PAR and MET which reproduced different composition ratios. The percent-

Table 4
Comparative studies for commercial tablets[a]

	Ratio spectra derivative	HPLC
Paracetamol (mean ± S.D.)[b]	299.5 ± 1.07 $t = 0.106$ (2.306)[c]	299.6 ± 1.82
Methocarbamol (mean ± S.D.)[b]	374.3 ± 0.88 $t = 2.094$ (2.306)[c]	375.3 ± 0.43

[a] Myorel tablets are the product of Sanofi-Doğu Pharm. Ind. (Turkey); each tablets was labeled to contain 300 and 375 mg of PAR and MET, respectively.
[b] Mean and standard deviation for five determinations.
[c] Values in parentheses are the theoretical values at $P = 0.05$.

age recoveries and their relative standard deviations (average of five determinations) were found to be 99.6 and 0.48% for PAR and 99.8 and 0.49% for MET, respectively.

3.3. Assay in tablet dosage form

When working on synthetic mixture, results encourage the use of the methods described for the assay of PAR and MET in commercial tablets.

The results obtained are shown in Table 4. The results were statistically compared using student t-test. As shown from the table, the calculated t-values were less than the theoretical value, indicating no significant difference between the two methods.

4. Conclusion

The reported methods for the simultaneous determination of PAR and MET in pharmaceutical formulations give accurate and precise results. The data obtained by both procedures are throughly comparable. The most striking feature of the ratio spectra derivative method is its simplicity and rapidity, no requiring time-consuming sample preparation such as filtration, degassing that are needed for HPLC procedure.

References

[1] O. Atay, M.T. Orbey, FABAD J. Pharm. Sci. 15 (1990) 223–230.
[2] S. Kir, C. Şafak, A. Türeli, A. Temizer, Fresenius J. Anal. Chem. 339 (1991) 264.
[3] G.R. Rao, A.B. Avadhanulu, D.K. Vatsa, A.R.R. Pantulu, Indian Drugs 27 (1990) 576–580.
[4] R.T. Sane, S.R. Surve, M.G. Gangrade, V.V. Bapat, N.L. Chonkar, Indian Drugs 30 (1993) 66–72.
[5] S.V. Erram, H.P. Tipnis, Indian Drugs 30 (1993) 116–119.
[6] F. Salinas, J.J. Berzas Nevado, A. Espinosa Mansilla, Talanta 37 (1990) 347–351.
[7] J.J. Berzas Nevado, C. Guiberteau Cabanillas, Anal. Lett. 23 (1990) 2077–2094.
[8] J.J. Berzas Nevado, C. Guiberteau Cabanillas, F. Salinas, Talanta 39 (1992) 547–553.
[9] R. Corbella Tena, M.A. Rodriquez Delgado, Mª.J. Sanchez, F. Garcia Montelongo, Talanta 44 (1997) 673–683.
[10] S.A. Özkan, N. Erk, Z. Şentürk, Anal. Lett. 32 (1999) 497–520.