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Simultaneous determination of some antiprotozoal drugs in different combined dosage forms by mean centering of ratio spectra and multivariate calibration with model updating methods

Eglal A Abdelaleem and Nada S Abdelwahab*

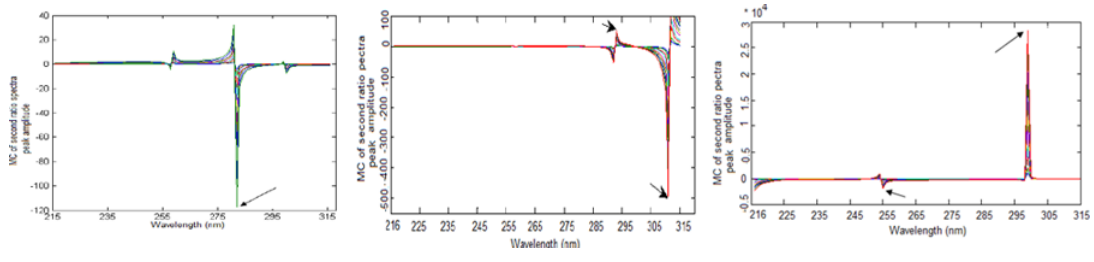
Abstract

Background: Metronidazole (MET) and Diloxanide Furoate (DF), act as antiprotozoal drugs, in their ternary mixtures with Mebeverine HCl (MEH), an effective antispasmodic drug. This work concerns with the development and validation of two simple, specific and cost effective methods mainly for simultaneous determination of the proposed ternary mixture. In addition, the developed multivariate calibration model has been updated to determine Metronidazole benzoate (METB) in its binary mixture with DF in Dimetrol® suspension.

Results: Method (I) is the mean centering of ratio spectra spectrophotometric method (MCR) that depends on using the mean centered ratio spectra in two successive steps that eliminates the derivative steps and therefore the signal to noise ratio is enhanced. The developed MCR method has been successfully applied for determination of MET, DF and MEH in different laboratory prepared mixtures and in tablets. Method (II) is the partial least square (PLS) multivariate calibration method that has been optimized for determination of MET, DF and MEH in Dimetrol ® tablets and by updating the developed model, it has been successfully used for prediction of binary mixtures of DF and Metronidazole Benzoate ester (METB) in Dimetrol ® suspension with good accuracy and precision without reconstruction of the calibration set.

Conclusion: The developed methods have been validated; accuracy, precision and specificity were found to be within the acceptable limits. Moreover results obtained by the suggested methods showed no significant difference when compared with those obtained by reported methods.

Graphical Abstract



Keywords: Mean centering of ratio spectra, PLS, Metronidazole, Diloxanide, Mebeverine and benzoate

* Correspondence: nadasayed2003@yahoo.com
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Bani-Sueif University, Alshaheed Shehata Ahmad Hegazy St., 62514 Beni-Suef, Egypt

Background

Mebeverine Hydrochloride (MEH), an effective antispasmodic drug that acts directly on the smooth muscle of gastrointestinal tract and is used in conditions such as irritable bowel syndrome [1]. DF, a luminal amoebicide, it is a dichloroacetamide derivative that principally acts in the bowel lumen and is used in the treatment of intestinal amoebicide [1]. Metronidazole (MET), an effective antiprotozoal drug, it is 5- nitroimidazole derivative with activity against anaerobic bacteria and protozoa [1] and is useful in both intestinal and extra intestinal amoebiasis. METB is a benzoyl ester of metronidazole that has the same action and uses [2,3], but mainly used in pediatric oral preparations because of the bland taste of the ester compared to the bitter taste of the free base [4]. Although both DF and MET act as antiprotozoal drugs, however their combination has a synergistic effect and acts on both vegetative and cystic forms of entamoeba histolytica [5].

Up to our knowledge, the literature survey revealed that none of the most recognized pharmacopoeies reported an analytical method for determination of the studied drugs in their ternary mixtures. On the other hand, only one journal published a report for determination of the studied mixture using TLC-Densitometric and derivative ratio spectrophotometric methods [6]. All other reported methods have been developed only for determination of each of the proposed drugs either alone or in combination with other drugs. British pharmacopoeia [7] reported non aqueous titration methods for determination each of the studied drugs in its dosage form; also USP [8] reported the same technique for determination of DF, MET and METB. MEH was recently determined by several techniques including, RP-HPLC [9-12], spectrophotometric, [13] and HPTLC [12] methods. MET was determined in plasma [14-16], blood [17] fish muscles [18], procine liver [19] and in different pharmaceutical preparations using different HPLC techniques, [20-23]. Also it has been determined by spectrophotometric [23,24] and voltametric methods [25]. Binary mixtures of DF and MET have been analyzed by RP-HPLC [5,26-28], spectrophotometric [5,28-30] and TLC-Densitometric techniques [5]. Due to the pharmaceutical importance of the studied drugs, this work aims to develop and validate accurate and precise methods for determination of MEH, DF and MET in Dimetrol® tablets, moreover, it aims to update the developed PLS model to be used for determination of DF and METB in Dimetrol® suspension without reconstruction of the calibration set. The developed MCR method has the advantages of being simpler and selective than the published spectrophotometric one and it does not need any derivatization steps, hence signal to noise ratio is enhanced. The developed methods do not need sophisticated instruments or any separation step and so can be used as alternative methods to LC methods in laboratories lacking the required facilities for these techniques.

Experimental

Instruments

A double beam UV-Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with 1 cm path length quartz cell is used and it is connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7. Matlab® version R2007b [31] was used for the proposed chemometric methods, the PLS was performed with PLS_Toolbox [32] for use with Matlab® R2007b.

Chemicals and reagents

Pure samples

Standards MEH, DF, MET and METB with claimed purities of 98.9, 100.5, 100.4 and 99.3%, respectively according to manufacturer certificate and were kindly donated by EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt).

Market sample

1-Dimetrol® film coated tablets batch No. 909537, were labeled to contain 375 mg MET, 250 mg DF and 50 mg MEH per tablet were manufactured by EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt.

2- Dimetrol® Suspension batch No. 906001 and 908403, was labeled to contain 200 mg METB and 100 mg DF per 5 mL suspension and was manufactured by EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt.

Methanol

HPLC grade (Sigma-Aldrich® Chemie GmbH, Germany).

Solutions

-Stock standard solutions of MEH, DF, MET and METB were prepared in methanol in the concentration of 1 mg mL^{-1}.

-Working standard solutions of MEH, DF, MET and METB were prepared in methanol in the concentration of 0.1 mg mL^{-1}.

Procedure

Mean centering of ratio spectra method (MCR)

Calibration model

Accurate aliquots equivalent to 10-250 μg each of MEH, DF and MET were transferred from their respective working standard solutions (0.1 mg mL^{-1}) into three series of 10-mLvolumetric flasks, the volume was completed to the mark with methanol to obtain final

concentration range of 1-25 μg mL^{-1} for each drug. The absorption spectra of the prepared solution were measured in the range of 216 - 315 nm.

For MEH: the recorded spectra were divided by standard spectrum of 6 μg mL^{-1} MET to obtain the first ratio spectra which was then mean centered. These vectors (mean centered ratio spectra) were then divided by the mean centered (MC) ratio of (α_{DF}/α_{MET}) corresponding to 6 μg mL^{-1} each, then the mean centering of the second ratio spectra were then obtained. *By the same way, the recorded spectra of DF* were divided by the standard spectrum of 20 μg mL^{-1} MET and the obtained ratio spectra were mean centered, these vectors were divided by the mean centered ratio of ($\alpha_{MEH}/\alpha_{MET}$) corresponding to 20 μg mL^{-1} each to obtain the second ratio spectra which were then mean centered. *For MET*, the scanned spectra of its prepared solutions were divided by the normalized spectrum of MEH and the obtained ratio spectra were mean centered. These vectors were divided by the mean centered ratio of (α_{DF}/α_{MEH}) corresponding to normalized spectrum each and the second ratio spectra were then mean centered.

The mean centered values of the second ratio spectra at 293-311 (peak to peak), 280 and 255-299 nm (peak to peak) for MEH, DF and MET, respectively were measured and plotted against the corresponding concentration of each drug to construct their respective calibration graphs.

Laboratory prepared mixtures

Different laboratory prepared mixtures containing different ratios each of MEH, DF and MET within their calibration ranges were prepared. The spectra of these mixtures were then recorded and the procedure under construction of calibration curves was then followed but using the recorded spectra of the prepared mixtures.

PLS model

Calibration model

Multilevel multifactor design was used for construction of the calibration and validation sets [33]. A five-level, three-factor calibration design was used in order to prepare 25 laboratory prepared mixtures containing different ratios of the three studied drugs, the concentrations details are given in Table 1. The absorption spectra of the prepared mixtures in the range of 230-300 nm were recorded; the spectral data acquisition was taken with 0.4 nm interval and then transferred to Matlab®R2007b for subsequent data manipulation. Seventeen mixtures were used for building the calibration model.

Assay of validation set

The remaining eight laboratory prepared mixtures, Table 1, were chosen to be used as an external validation set and the procedure given under construction of calibration set was then followed. The concentrations of each component were calculated using the optimized PLS calibration model.

Table 1 Concentrations of Mebeverine HCl, Diloxanide Furoate and Metronidazole in the calibration and validation sets

Sample No.	MEH (μg mL^{-1})	DF (μg mL^{-1})	MET (μg mL^{-1})
1	3	11	15
2	3	7	25
3	2	15	25
4*	2	11	20
5	4	15	20
6	4	13	25
7*	3	13	10
8	3.5	15	15
9	4	11	10
10	3	9	20
11	2.5	13	20
12	3.5	13	5
13*	3.5	9	10
14*	2.5	9	25
15	2.5	7	15
16	3.5	7	20
17*	2.5	11	5
18	2	13	15
19	3.5	11	25
20	3	15	5
21	4	7	5
22*	2.5	15	10
23	2	7	20
24*	4	9	15
25	2	9	5

* Samples used for model validation

Model updating

To perform model updating, the optimized PLS calibration set was augmented with different samples of Dimetrol® syrup containing known amounts each of DF and METB. One to six samples containing different concentrations of each were added to the initial calibration set and the predictive ability of the updated model was checked using external validation samples (prepared from Dimetrol® suspension with different batch number) their concentrations were previously determined using the reported RP-HPLC method [29]. The RMSEP values were then calculated for each component using the developed model with different numbers of the added updating samples.

Application to market samples

Dimetrol® tablets

The content of twenty coated tablets of Dimetrol® was separately weighed. An accurately weighted portion

equivalent to 150 mg MET, 100 mg DF and 20 mg MEH were separately transferred into 100-mL calibrated measuring flask and then 75 mL methanol was added. The prepared solution was sonicated for 30 minutes, the volume was completed with methanol and the solution was then filtered. Appropriate dilution of the prepared solution was made to prepare the working solution (0.1 mg mL^{-1}) of DF and the corresponding concentration of MET and MEH (0.15 and 0.02 mg mL^{-1}, respectively).

Dimetrol® suspension

5 mL of Dimetrol® suspension was accurately transferred (after vigorous shaking) into a 100 mL measuring flask, sonicated in 50 mL methanol for 10 min and filtered into another 100 mL volumetric flask. The residue was washed using (3 × 10) mL methanol and the volume was completed to the mark with methanol. Appropriate dilutions of the prepared solution were made to prepare the working solution (0.1 mg mL^{-1}) of DF and the corresponding concentration of METB.

Results and Discussion

The UV absorption spectra of MEH, DF, MET and METB, Figure 1, displays considerable overlap, where the application of conventional spectrophotometry, its direct derivative and derivative ratio techniques failed to resolve these overlapping.

Mean centering of ratio spectra spectrophotometric method (MCR)

The developed MCR method depends on the mean centering of ratio spectra, it eliminates the derivative steps and therefore signal-to-noise ratio is enhanced [34] and it has been applied for resolving binary and ternary mixtures in complex samples with unknown matrices [35]. The mathematical explanation of the method was illustrated by Afkhami and Bahram [34-36].

In order to optimize the developed MCR method, effect of divisor concentration on the selectivity of the method has been tested. Different concentrations each of MEH, DF and MET (normalized spectrum, 4, 6, 8, 14 and 20 μg mL^{-1}) were tested. It was found that the divisor had a great effect on the selectivity of determination of MEH and DF where reproducible and good results have been obtained upon using concentration of 6 μg mL^{-1} each of MET and DF (for MEH) and 20 μg mL^{-1} each of MET and MEH (for DF) as divisors. On the other hand, changing the concentration of the divisor had no significant effect on the specificity of MET determination, therefore, normalized spectrum each of MEH and DF was used as a divisor.

Beer's Lambert's law has been obeyed in the range of 1-24, 2-25 and 1-24 μg mL^{-1} for MEH, DF and MET, respectively, Figure 2. Calibration curves relating the mean centered values at 293-311 (peak to peak)), 280 and 255-299 nm (peak to peak) to the corresponding concentrations of MEH, DF and MET, respectively have been constructed from which the regression equation parameters found in Table 2 have been obtained. Specificity of the method has been validated by application on different synthetic mixtures containing different ratios of the three studied drugs where good percentage recoveries with law RSD% values were obtained, Table

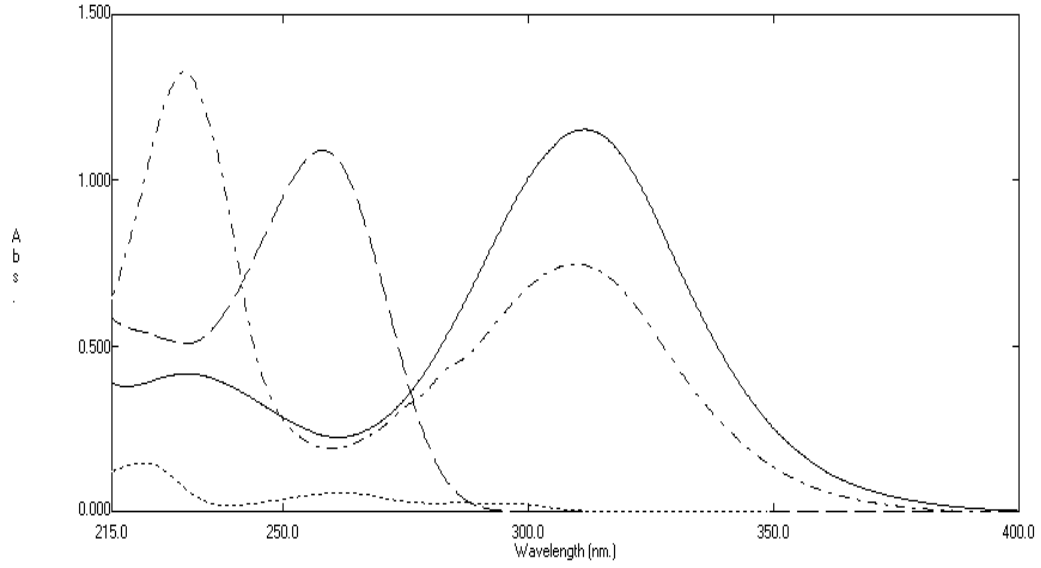


Figure 1 Zero order absorption spectra of 22.5 μg mL^{-1} MET (), 15 μg mL^{-1} DF (————), 3 μg mL^{-1} MEH (.....) and 22.5 μg mL^{-1} METB (-.-.-.) in methanol.

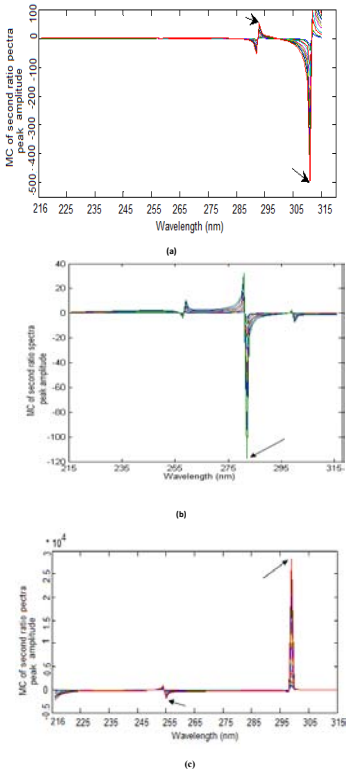


Figure 2 The mean centered second ratio absorption spectra of)a) MEH, (b) DF and (c) MET in methanol.

3. Moreover, accuracy of the suggested method has been confirmed by its application for determination of blind samples of pure drugs and good results were obtained, Table 2. The results obtained on applying the developed MCR method for determination of pure MEH, DF and MET on different days confirmed the precision of the method, Table 2.

Table 2 Regression and analytical parameters of the proposed MCR method for determination of Mebeverine HCl, Diloxanide Furoate and Metronidazole

Parameters	MCR Method		
	MEH	DF	MET
Calibration range	1-24	2-25	1-24
	μgmL^{-1}	μgmL^{-1}	μgmL^{-1}
Slope	22.726	4.6825	1095
Intercept	6.136	0.4945	-6.5508
Correlation coefficient	0.9998	0.9999	0.9999
Accuracy	99.13	100.12	100.50
Precision			
Repeatability	0.991	0.879	0.799
Intermediate precision	1.205	1.003	1.220

Partial least squares (PLS)

Recently quantitative spectroscopy has been greatly improved by the use of variety of multivariate statistical methods [37-39]. This work aims to investigate the ability of PLS model to quantify each of MEH, DF and MET with overlapping UV spectra, Figure 1, in Dimetrol® tablets and to apply the optimized PLS model for determination of DF and METB in Dimetrol® suspension by performing model updating.

The first step in the determination of the cited drugs by multivariate calibration method is to construct the calibration matrix for the ternary mixture (MEH, DF and MET), calibration set was obtained by using the absorption spectra of seventeen laboratory prepared mixtures (using five level, three-factor calibration design) of different ratios each of MEH, DF and MET in the range of 230-300 nm and the spectral data acquisition was taken with 0.4 nm interval. The regions from 200 nm- 229 nm and above 300 nm were rejected. In this study, the data was auto scaled as a pre- processing step, leave one out cross validation method was applied and the root mean squared error of calibration RMSEC values of the initially developed models were compared. The selected model was that with the smallest number of factors such that RMSEC for that model was not significantly greater than RMSEC from model with an additional factor. Four factors were selected by F-test for PLS [40]. The second step is to assess the prediction ability of the suggested model, an external validation set consisting of different eight prepared mixtures was used and the root mean squared error of prediction (RMSEP) values were calculated, Table 3, where the results obtained indicated the high predictive ability of the developed model.

In order to apply the developed method for determination of DF and METB in Dimetrol® suspension model updating was performed.

Model-updating

Model updating [41] means predicting a new component without reconstruction of calibration set. In this work the model-updating was applied to predict the concentration of the modeled compound, DF, in another dosage form which had different spectral characteristic, Figure 3, that contains this drug in addition to an unmodeled one (METB) without reconstruction of the calibration set.

The minimum number of samples needed to efficiently update the developed model must be accurately determined and the influence of number of samples added to the calibration set on RMSEP was studied for the developed multivariate calibration model. The number of different samples added shows a large impact on the predictive ability of the updated model as shown in

Table 3 Determination of the studied drugs in the laboratory prepared mixtures (L.P. M) and tablets by the proposed methods and statistical comparison with the reported method

Parameters	MCR method			PLS method		
	MEH	DF	MET	MEH	DF	MET
L.P.M[a]	99.91 ± 2.338	100.88 ± 2.149	102.39 ± 1.780	99.85 ± 2.368	101.40 ± 1.311	100.54 ± 2.194
RMSEP				0.063	0.200	0.459
Dimetrol® tablets[b] (B.No.909537)	98.49 ± 1.419	100.15 ± 1.332	99.45 ± 1.127	99.33 ± 1.329	99.44 ± 0.965	100.78 ± 1.575
Standard addition[a]	99.23 ± 1.122	101.50 ± 2.701	100.02 ± 1.752	102.54 ± 0.638	100.00 ± 1.858	98.90 ± 0.996
Degree of freedom	10	10	10	10	10	10
F-test						
(5.050)[c]	2.037	2.506	1.061	1.786	1.315	2.070
Degree of freedom Student's -t test (2.228)[c]	10	10	10	10	10	10
	1.905	1.137	0.376	0.755	0.758	2.007

[a]: Average of 3 determinations

[b]: Average of 6 determinations

[c]: The values in the parenthesis are the corresponding theoretical values at p = 0.05

Table 4. Three samples were found to be necessary to perform an efficient update of the developed PLS model. The results of analysis of Dimetrol® suspension by the updated model are presented in Table 5.

The two developed methods are valid and applicable for the analysis of the studied drugs in Dimetrol® tablets and suspension. The validity of the methods was assessed by applying the standard addition technique, Tables 3 and 4, which showed that the developed methods are accurate and specific for determination of the cited drugs in presence of dosage form excipients. The results obtained by the developed methods for analysis of Dimetrol ® tablets and suspension have been compared to those obtained by applying the reported methods [6,29] using Student's-t and F tests and no significant difference was found, Tables 3 and 4.

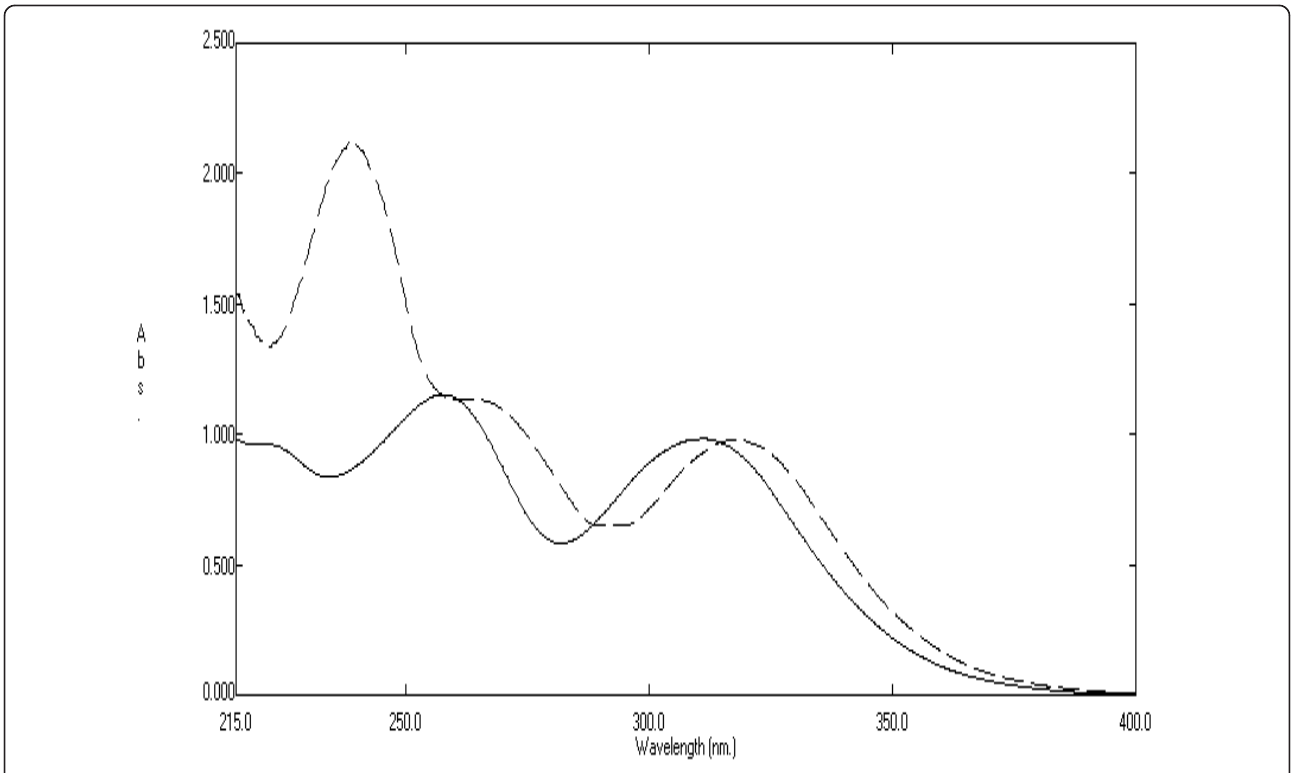


Figure 3 The average spectra of Dimetrol ® tablets (- - - - -) and Dimetrol ® suspension (____) in methanol.

Table 4 RMSEP and percentage recoveries of the updated PLS model at different number of updating samples

No of updating samples	DF		METB	
	RMSEP	% improvement	RMSEP	% improvement
1	0.923		1.018	
2	0.393	57.42%	0.535	47.45%
3*	0.309	66.52%	0.356	65.03%
4	0.305	66.96%	0.377	62.97%

* The optimum number of updating samples needed

Table 5 Determination of the studied drugs in Dimetrol® suspension by the updated PLS model and statistical comparison with the reported RP-HPLC method

Parameters	Updated PLS model	
	DF	METB
Dimetrol® suspension[a] (B.No.906001)	101.19 ± 1.327	99.59 ± 0.981
Standard addition[b]	99.95 ± 1.233	100.55 ± 1.159
Degree of freedom F-test (5.050)[c]	10	10
	0.738	1.026
Degree of freedom	10	10
Student's -t test (2.228)[c]	0.200	0.444

[a]: Average of 6 determinations

[b]: Average of 3 determinations

[c]: The values in the parenthesis are the corresponding theoretical values at p = 0.05

Conclusion

The presented methods are accurate, precise and sensitive which are applicable for simultaneous determination of MEH, DF and MET. The developed MCR method has the advantages over the published methods in being more simple, rapid and the data processing step is not time consuming as it does not need the application of complex algorithms. Also, it does not need derivative steps and so signal to noise ratio has enhanced. On the other hand, the developed PLS model has the advantages of being cost and time effective because only one model has been used for resolution of the studied ternary mixture and then by updating the model, it could be successfully applied for determination of DF and METB binary mixture in another dosage form.

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Authors' contributions

EAA and NSA carried out all the experimental work, designed the proposed methods and analyzed the data statistically together. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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