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# SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF TWO COFORMULATED DRUGS WITH HIGHLY DIFFERENT CONCENTRATIONS. APPLICATION ON VILDAGLIPTIN AND METFORMIN HYDROCHLORIDE

**H. E. Zaazaa, E. S. Elzanfaly, A. T. Soudi,* and M. Y. Salem**

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*A new smart simple validated spectrophotometric method was developed for the determination of two drugs one of which is in a very low concentration compared to the other. The method is based on spiking and dilution then simple mathematical manipulation of the absorbance spectra. This method was applied for the determination of a binary mixture of vildagliptin and metformin hydrochloride in the ratio 50:850 in laboratory prepared mixtures containing both drugs in this ratio and in pharmaceutical dosage form with good recoveries. The developed method was validated according to ICH guidelines and can be used for routine quality control testing.*

***Keywords:*** *vildagliptin, metformin HCl, first derivative, spiking and dilution, spectrophotometric method.*

**Introduction.** Several binary dosage forms contain one component in a very high concentration when compared to the other. Using direct spectrophotometry, it is usually impossible to determine the component of lower concentration especially when it has low absorpitivity. The aim of this work was to develop a method that allows the determination of the components of a binary mixture when one of them is present in a very low concentration compared to the other. The method was applied for the determination of vildagliptin and metformin hydrochloride in pure powder and in pharmaceutical formulation where the concentration of vildagliptine is 17 times less than that of metformin.

Vildagliptin (VID) is chemically *S*-1-[*N*-(3-hydroxyl-1-adamantyl)glycyl]pyrrolidine-2-carbonitrile. It is a potent dipeptidyl peptidase inhibitor used in the treatment of type II diabetes [1]. Metformin hydrochloride (MET) is chemically *N*,*N*-dimethylimidodicarbonimidic diamide. It is used as oral anti-hyperglycemic drug [1].

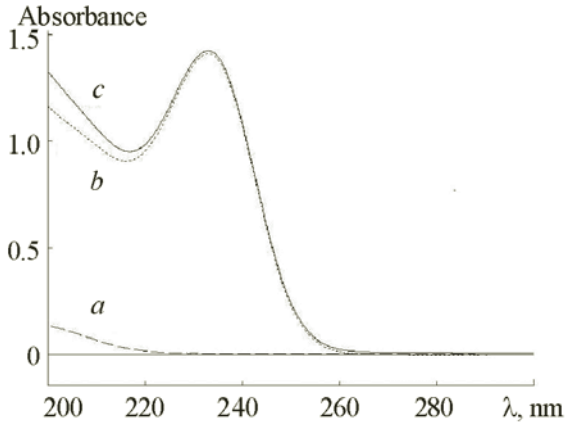
Vildagliptin and metformin HCl were simultaneously determined by HPLC [1–7] and capillary electrophoresis [8], and only two spectrophotometric methods were reported for the determination of this mixture. The first method was reported by Gundala et al. [9] where the drugs were determined at 217 and 234 nm and nothing was mentioned about the interferences due to overlapped spectra. The second method was reported by Shrikrishna et al. [10] and depends on the use of Vierordt's equation for the simultaneous estimation of the drugs in ratio 1:10 for VID and MET, respectively. This work aims to present a simple and rapid spectrophotometric method for the simultaneous determination of VID and MET in their combined dosage form in the ratio 1:17.

**Theory.** When two drugs *A* and *B* are co-formulated in highly different concentrations such that *A* is considered a minor constituent and the concentration of *B* is much higher and the spectrum of B completely overshadows the spectrum of *A*, this problem can be solved by spiking the mixture with a known constant concentration of *A* followed by dilution of the mixture. This process increases the concentration of *A* and decreases that of *B* and we are left with a binary mixture that can be determined by any of the methods used for binary mixtures.

This can be represented by the following equations. Let the drug present in small concentration (minor constituent) = *A*, the drug present in much higher concentration = *B*, and the binary mixture = $A + B$. By adding a known constant amount of *A* (such as 4*A*), we have $(A + 4A) + B$. This step is then followed by dilution (5 times, for example) $(5A + B)/5 = A + B/5$.

*To whom correspondence should be addressed.

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El- Aini Str., Cairo 11562, Egypt; e-mail: aya.soudi@pharma.cu.edu.eg. Published in Zhurnal Prikladnoi Spektroskopii, Vol. 83, No. 1, pp. 154–157, January–February, 2016. Original article submitted March 27, 2015.



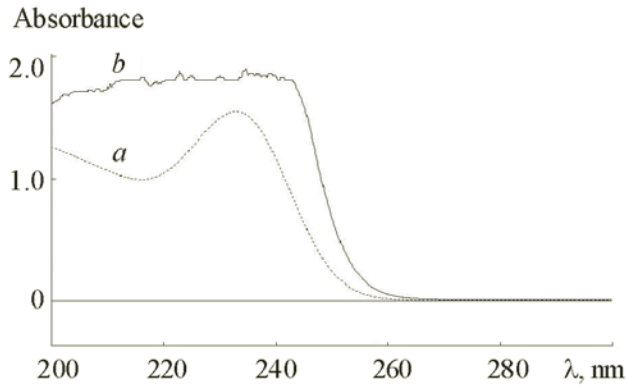




Fig. 1. Absorption spectra of 5 μg/mL VID (a), 17 μg/mL MET (b) and a mixture of 5 and 17 μg/mL VID and MET (c).

Fig. 2. Absorption spectra of mixture of 5 and 25 μg/mL VID and MET (a) and 5 and 50 μg/mL VID and MET (b).

Thus the concentration of *B* in the binary mixture is reduced to a level that allows the simultaneous determination of *A* and *B* by the usual spectrophotometric methods used for binary mixtures such as derivative, derivative ratio spectrophotometry, ratio difference, etc.

**Materials and Method.** A Shimadzu UV-1650 dual beam UV-visible spectrophotometer (Japan) was used. Metformin working standard was kindly supplied by Chemical industries development CID company, Cairo, Egypt, and its purity was reported to be 99.8%. Vildagliptin working standard was purchased from Shanghai Richem international company, China, and its purity was reported to be 99.4%. Stock solutions were prepared as 1 mg/mL in double distilled water for both drugs. Working solutions were prepared as 50 μg/mL in double distilled water for both drugs. Galvusmet® tablets (label claim: 850 mg metformin hydrochloride and 50 mg vildagliptine per tablet) manufactured by Novartis International Company, Cairo, Egypt, were purchased from the local market.

Aliquots (1–10 mL) of VID and (0.4–4 mL) of MET were transferred from their corresponding working solutions to separate 10-mL volumetric flasks, and the volumes were completed with double distilled water. The absorption spectra were scanned and then the first derivatives were obtained with scaling factor 10 and $\Delta\lambda = 2$. The peak amplitudes at 216 nm for VID and at 246 nm for MET were obtained, and the two calibration curves relating the peak amplitudes and the corresponding concentrations were constructed.

The laboratory mixture was prepared to contain 25 μg/mL VID {5+20} with 85 μg/mL MET. The solution was diluted 5 times to obtain 5 μg/mL VID (*A*) with 17 μg/mL MET (*B*). The absorbance spectrum of the mixture was obtained then derivative was computed. The peak amplitudes at 216 and 246 nm were measured, and the concentrations of VID and MET were determined from their regression equations, respectively.

Ten tablets were weighed, finely powdered, and the weight of one tablet was transferred to a 100-mL volumetric flask. Fifty milliliters of methanol was added and the whole sonicated for 20 min. The volume was completed with double distilled water then the solution was filtered. One mL of the filtrate and 2 mL of stock VID were added to 100-mL volumetric flask and the volume was completed with water. The resulting solution was diluted 5 times to obtain (5 μg/mL VID and 17 μg/mL MET) and the procedure was completed as above.

**Results and Discussion.** Drugs co-formulated in abnormal ratios offer great challenges in their simultaneous determination; this work represents a method to overcome such a difficulty.

VID and MET were co-formulated in a ratio 50:850. This mixture was chosen as a model to represent the suggested method. With such a ratio, VID is the minor component. It also has low absorpitivity and cannot be quantified. This work was designed to develop a new, simple, and accurate spectrophotometric method for the simultaneous determination of VID and MET in the presence of each other and in their pharmaceutical formulations without complexation or derivatization but with simple practical processing and manipulations of spectral data.

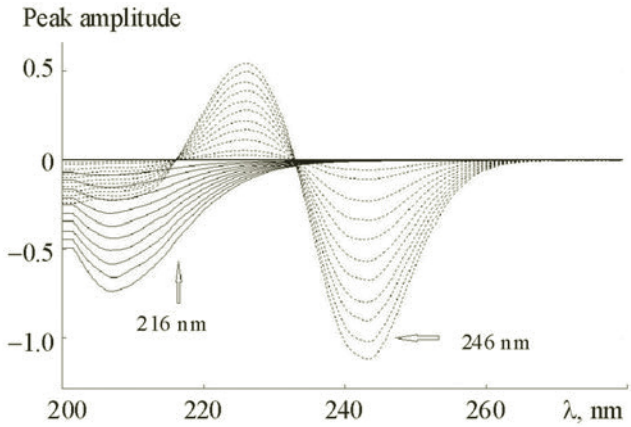



Fig. 3. First derivative spectra of 5–50 μg/mL VID (solid lines) and 1–20 μg/mL MET (dotted lines).

TABLE 1. Validation Parameters of the Proposed Method for the Determination of Vildagliptin and Metformin

| Parameter | VID | MET |
|---|---|---|
| Linearity | | |
| Range, μg/mL | 5–50 | 1–20 |
| Correlation coefficient $r^2$ | 0.9993 | 0.9998 |
| Slope | 0.0091 | 0.0496 |
| Intercept | –0.0033 | 0.0028 |
| Accuracy (mean ± SD) | | |
| Low concentration | 101.03 ± 1.27 | 99.76 ± 0.15 |
| Medium conc. | 100.06 ± 0.25 | 100.79 ± 0.17 |
| High conc. | 101.46 ± 0.13 | 99.38 ± 0.06 |
| Precision | | |
| Repeatability[a] | 99.18 ± 0.60 | 100.16 ± 1.57 |
| Intermediate precision[b] | 99.20 ± 0.32 | 100.54 ± 0.81 |
| Specificity | 101.03 ± 1.27 | 98.73 ± 0.38 |

[a] Intra-day precision ($n$ = 3), average of three concentrations repeated three times within the same day.
[b] Inter-day precision ($n$ = 3), average of three concentrations repeated three times on three consecutive days.

The absorption spectra of these drugs show that MET has high absorbitivity with absorption maxima at 233 nm, while VID has low absorbitivity with no absorption maximum (Fig. 1). This fact together with the presence of MET high concentration (17 times more than VID) makes the spectrum of VID completely overlapped by that of MET (Fig. 1). Furthermore, at much higher concentration of MET, no absorption spectrum can be recorded for the mixture (Fig. 2). Experimentally, it was found that to determine VID in the presence of MET, the latter's concentration should not exceed five times the concentration of VID. It is therefore necessary to increase VID concentration by spiking to reach a ratio 1:5 for VID and MET, where a good absorbance spectrum can be obtained. This spectrum enables the simultaneous determination of both drugs by any of the spectrophotometric methods used for binary mixtures. Derivative spectrophotometry was chosen as it is the simplest of all, but further investigations showed that this mixture can also be resolved by derivative ratio and ratio difference spectrophotometric methods.

TABLE 2. Determination of Vildagliptin and Metformin in Galvusmet® Tablets (label claim: 850 mg metformin hydrochloride and 50 mg vildagliptine) by the Proposed Method and Application of Standard Addition Technique

| Proposed method | Standard addition | | | | |
|---|---|---|---|---|---|
| % Recovery | Taken | Added | Standard found, μg | % Recovery | Mean % Recovery |
| VID<br>100.29 ± 1.27 | 5 μg | 5 μg<br>(100%) | 5.09<br>4.98<br>4.98 | 101.76<br>99.56<br>99.56 | 100.29 ± 1.27 |
| MET<br>100.95 ± 0.52 | 17 μg | 2 μg<br>(11.76%) | 1.98<br>1.96<br>1.98 | 98.99<br>97.98<br>98.99 | 98.66 ± 0.58 |

Thus, the first derivative spectra were obtained using $\Delta\lambda$ = 2 nm and scaling factor of 10, the peak amplitude was measured at 216 nm for VID and 246 nm for MET, and the concentration of both drugs was calculated from the corresponding regression equations. (Fig. 3)

The suggested method was validated according to ICH guidelines as shown in Table 1. The linearity ranges for VID and MET were found to be 5–50 and 1–20 μg/mL with correlation coefficients of 0.9993 and 0.9998, respectively. The values of the correlation coefficients and the accuracy and precision show that derivative spectrophotometry successfully overcomes the small contribution from the spectrum of VID at the $\lambda_{max}$ of MET in the zero-order absorbance spectrum and also eliminates the interference of MET by measurement of the peak amplitude at the zero crossing at 216 nm.

The proposed method was applied to Galvusmet® tablets, and satisfactory results were obtained for VID and MET as shown in Table 2. Application of the standard addition technique revealed that there are no interferences from the excipients in the dosage form (Table 2).

**Conclusions.** From the data obtained it is proved that determination of drugs formulated together in abnormal ratios can be done by simple practical processing (spiking followed by dilution) followed by manipulation of spectral data. The proposed method was successfully applied to the determination of VID and MET coformulated in a ratio 1:17 and proved to be accurate, precise and specific over the specified range and can be used for quality control and routine analysis of VID and MET in pure powder and pharmaceutical formulations.

The advantage of the proposed method is the simplicity and its accuracy in the determination of the minor component without complexation or derivatization.

## REFERENCES


1. B. M. Ishaq, D. K. V. Prakash, and D. G. K. Mohan, *J. Global Trends Pharm. Sci*., **3**, 747–754 (2012).
2. M. Attimarad, S. H. Nagaraja, B. E. Aldhubaib, and A. Al-Najjar, *J. Young Pharm*., **6**, 40–46 (2014).
3. R. Pontarolo, A. Gimenez, T. Francisco, R. Ribeiro, F. Pontes, and J. Gasparetto, *J. Chromatogr*. *B*, **15**, 133–140 (2014).
4. C. P. Uber, F. L. D. Pontes, J. C. Gasparetto, T. M. G. D. Francisco, M. S. Piantavini, M. A. Cardoso, and R. Pontarolo, *Int. J. Pharm. Pharm. Sci*., **6** (2014).
5. P. S. R. C. N. P. Varma, D. A. Lakshmana, and S. C. Dinda, *Int. Res. J. Pharm*., **4**, 2230–8407 (2012).
6. B. Santhosha, A. Ravindranath, and C. Sundari, *Int. Res. J. Pharm. Appl. Sci*., **2**, 22–28 (2012).
7. K. P. latha and D. Ramachandran, *Int. J. Pharm. Pharm. Sci*., **5**, 459–463 (2013).
8. A. T. Barden, B. L. Piccoli, N. M. Volpatoa, and M. Steppea, *Anal. Methods*, **5**, 5701–5708 (2013).
9. U. Gundala, C. S. Bhuvanagiri, and D. Nayakanti, *Am. J. Pharm. Tech. Res.*, **3**, 338–345 (2013).
10. B. Shrikrishna, S. V. Mulgund, and N. S. Ranpice, *Pharma Chem*., **5**, 24–27 (2013).