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Original Article

RP-UPLC method development and validation for simultaneous estimation of vildagliptin with metformin hydrochloride and ciprofloxacin hydrochloride with dexamethasone sodium phosphate

Manal Mohamed Fouad, Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt and October University for Modern Sciences and Arts, 6 October City, Egypt

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ABSTRACT

A novel, sensitive and rapid assay method using Ultra Performance Liquid Chromatography (UPLC) has been developed and validated as per ICH guideline for simultaneous determination of two binary mixtures; vildagliptin with metformin hydrochloride and ciprofloxacin hydrochloride with dexamethasone sodium phosphate. Chromatographic separation was achieved on a Phenomenex C_{18} column (100 mm, 2.1 mm i.d., 1.8 µm) using an isocratic method with mobile phase of potassium di-hydrogen phosphate (pH 4): acetonitrile in the ratio 70:30 (v/v) for vildagliptin/metformin mixture or 80:20 (v/v) for ciprofloxacin/dexamethasone mixture. The flow rate was 1 mL/min, temperature of the column was maintained at ambient and detection was made at 220 or 254 nm for the two mixtures, simultaneously. Linearity studies indicated that the drugs obeys Beer's law over the range of 0.5-5 µg/mL for vildagliptin, 5-50 µg/mL for metformin hydrochloride while ranges from 2-20 µg/mL for both ciprofloxacin hydrochloride and dexamethasone sodium phosphate. The proposed method is precise, accurate, linear, robust and fast. The short retention time allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

Keywords: UPLC, Simultaneous estimation, Vildagliptin, Metformin hydrochloride, Ciprofloxacin hydrochloride, Dexamethasone sodium phosphate

INTRODUCTION

Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products. Though high-performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients and dosage forms, it is often a slow technique because of the complexity of some of the samples. A new category of separation technique, ultra-performance liquid chromatography (UPLC), has proven to be one of the most promising developments in the area of fast chromatographic separations with its unique characteristics of high chromatographic resolution, speed, and sensitivity analysis. In the present work, this technology has been applied to the method development and validation study of binary mixtures of vildagliptin with metformin hydrochloride and ciprofloxacin hydrochloride with dexamethasone sodium phosphate.

Vildagliptin (VLD); 1-[(3-hydroxy-adamant-1-ylamino) acetyl]-pyrrolidine-2(S)-carbonitrile is a highly selective dipeptidyl peptidase-4(DPP-4) inhibitor used as an oral anti-hyperglycemic drug [1]. Metformin hydrochloride (MET); 3-(diaminomethylidene)-1,1-dimethylguanidine is a biguanide anti-hyperglycemic agent, acting by inhibiting gluconeogenesis and thereby suppressing hepatic glucose release [2]. The combination of metformin and vildagliptin offers advantages as being compared to currently used combinations with additive efficacy and complimentary mechanisms of action; therefore, by specifically combining these agents in a single tablet, there is considerable potential to achieve better blood glucose control and to improve compliance to therapy [3]. Literature survey revealed that some analytical methods such as spectroscopy [4-7], HPLC [8-17] and LC-MS [18,19] have been reported for the estimation of vildagliptin and metformin individually, in combination with each other or with other drugs.

***Corresponding Author Address:** Manal Mohamed Fouad, Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt and October University for Modern Sciences and Arts, 6 October City, Egypt*

Ciprofloxacin hydrochloride (CFH); [1-cyclopropyl- 6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid hydrochloride monohydrate] is a synthetic antibiotic of the fluoroquinolone drug class [20]. Dexamethasone sodium phosphate (DSP); [9-fluoro-11b, 17, 21-trihydroxy-16α- methylpregna-1, 4- diene-3, 20-dione 21-(dihydrogen phosphate) disodium salt] is a highly selective glucocorticoid which is widely used in ocular inflammatory diseases [21]. Dexamethasone in combination with ciprofloxacin hydrochloride is used in several anti-infective eye preparations to treat acute and sub-acute conjunctivitis, keratitis and corneal ulcers [22]. CFH was determined individually by non-aqueous titration [23], UV-spectrophotometry [24], colorimetry [25-27], HPLC [28-31], TLC [32,33], gas chromatography [34] and capillary electrophoresis [35,36]. On the other hand, DSP was determined individually by UV spectroscopy [37], HPLC [38-41], TLC [42] and gas chromatography[43].Meanwhile, a few spectrophotometric [44,45], HPLC [46,47] and HPTLC [48] methods were reported for the simultaneous determination of both drugs

Though the literature provides different methods for the determination of the cited mixtures, yet there were no reports available on their estimation by UPLC method. It is, therefore, felt necessary to develop a new rapid method for the separation and quantification of the studied drugs in their combination using an isocratic mobile phase with UPLC system.

EXPERIMENTAL

Instrumentation: Chromatographic separation was performed on an Agilent 1290 UPLC (USA) with binary pump and UV detector. Analysis was performed on a Phenomenex C $_{18}$ column (100 mm, 2.1mm i.d., 1.8 µm) using an autosampler maintained at room temperature. Data acquisition and processing was performed using EMPOWER (2) software. An Elma S100 ultrasonic processor (Germany) was used for the degassing of the mobile phase.

Materials and reagents: ***Pure samples:*** Vildagliptin (99.98%) and metformin hydrochloride (100.01%) working standards were kindly supplied from Egyptian International Pharmaceutical Co. (EIPICO, Egypt). Ciprofloxacin hydrochloride and dexamethasone sodium phosphate were obtained from Grand Pharma pharmaceutical company (10th of Ramadan, Egypt) with claimed purity of 100.41 and 100.01 %, respectively as stated by the manufacturer.

Market samples: Galvus Met®, Novartis Corporation, Egypt) labeled to contain 50 mg VLD and 500 mg MET. Peopo-otic® ear drops (Grand Pharma for Glen mark pharmaceuticals, Egypt) containing 0.3% CFH and 0.1% DSP, purchased from local pharmacy.

Solvents: Ortho-phosphoric acid (Sigma –Aldrich, Germany), acetonitrile HPLC grade (Fisher, UK), potassium dihydrogen phosphate (ADWIC Chemicals, Egypt). Bi-distilled water was prepared and used throughout the procedure.

Preparation of solutions:

Preparation of phosphate buffer: 7.0 g potassium dihydrogen phosphate were weighed into a 1000-mL beaker, dissolved and diluted to 1000 mL with water and finally adjusted to pH 4.0 using ortho-phosphoric acid.

Preparation of mobile phase: A mixture of the above buffer and acetonitrile in their appropriate ratio was prepared, degased in ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum filtration.

Standard solution preparation: 50 mg VLD and 500 mg MET standards were accurately transferred into separate 100-mL volumetric flasks to which about 20 mL of methanol were added and sonicated then diluted to the mark with the same solvent (stock solution). Further dilution was made by pipetting 2 mL of each of the above stock solutions into a 100-mL volumetric flask and dilution up to the mark the mobile phase to obtain a solution containing 0.01 mg/ mL VLD and 0.1 mg/ mL MET.

Stock solutions of CFH and DSP (1 mg/mL) were prepared by dissolving 100 mg of each drug in distilled water in separate 100- mL measuring flasks, then adjusting to volume. Working solutions were freshly prepared by diluting the stock solutions with the mobile phase to obtain a solution containing 0.1 mg/mL of each drug.

Sample solution preparation: Ten Galvus-Met® tablets were weighed separately and crushed. Amount of powder equivalent to 10 mg VLD and 100 mg MET were weighed, transferred into 100-mL volumetric flask and treated with 25 mL methanol. Volumetric flask was sonicated for 15 min, adjusted to volume with methanol and filtered through 0.45-micron membrane filter paper. The resulting solution was further diluted with mobile phase to get a final concentration of 0.01 mg/ mL VLD and 0.1 mg/ mL MET.

Sample solution of CFH and DSP was prepared by transferring 5.0 mL of Peopo-otic® ear drops into a 50-mL volumetric flask. Volume was made up to the mark with the mobile phase to give a concentration of 0.3 mg/ mL CFH and 0.1 mg/ mL of DSP.

Chromatographic conditions: The analysis was achieved on a Phenomenex C_{18} column (100 mm, 2.1mm i.d., 1.8 µm, USA). Isocratic elution was performed using a mobile phase of potassium di-hydrogen phosphate (adjused to pH 4 using Ortho-phosphoric acid): acetonitrile in the ratio 70:30 v/v for VLD/MET mixture or 80:20 v/v for CFH/DSP mixture at a flow rate of 1 mL/min. The detection was monitored at the wavelength of 220 nm or 254 nm for the two mixtures, simultaneously. Analysis was performed at ambient temperature, the injection volume was 10.0 µL and a chromatographic runtime of 10 min was used.

Method validation

The method was validated in accordance with ICH guidelines [49].

Linearity: Linearity of the method was studied by injecting five concentrations of the drugs in triplicate prepared in the range of 0.5 -5 µg/mL for VLD, 5- 50 µg/mL for MET and 2-20 µg/mL for CFH and DSP into the UPLC system. Linear graphs were plotted by using the peak areas against concentration in µg/mL from which the correlation coefficients, slopes and *Y*-intercepts of the calibration curves were determined.

Accuracy: The accuracy of the method was determined by calculating the recoveries of VLD, MET, CFH and DSP by the standard addition method. Known amounts of standard solutions were added to prequantified sample solutions. The chromatograms were recorded and the % recovery was calculated from regression equations of the calibration curves.

Precision: It was determined by assay of sample solutions (three different concentrations) three times in a day for intraday precision and for three different days for interday precision.

Limit of detection and Limit of quantification: The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

Specificity: In the present work, specificity was checked by analyzing VLD with MET and CFH with DSP in their laboratory prepared mixtures containing different ratios of the cited drugs within the linearity range. The concentration of each drug was calculated by substitution in the corresponding regression equation, from which mean % recovery can be calculated.

Robustness: To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, wavelength, and mobile phase composition). Changes in the chromatographic parameters were evaluated for the studies.

RESULTS AND DISCUSSION

In the present work, UPLC technology has been applied to the method development and validation study of binary mixtures of vildagliptin with metformin and ciprofloxacin with dexamethasone.

Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition and pH, wavelength of detection, column and diluents were optimized. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Phenomenex C_{18} column (100 mm, 2.1mm i.d., 1.8 µm) was used for the elution. Several proportions of solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Buffers like potassium di-hydrogen phosphate, di-potassium hydrogen phosphate, and di-sodium hydrogen phosphate were tried; potassium di-hydrogen phosphate (adjusted to pH 4 using Ortho-phosphoric acid): acetonitrile in the ratio 70:30 (v/v) for VLD/MET mixture or 80:20 (v/v) for CFH/DSP mixture gave perfectly eluted peaks. Trials were also done using different flow rates and detection was done at different wavelengths, in which 1 mL/min was optimum for separating both mixtures at a detection wavelength of 220 or 254 nm for VLD/MET and CFH/DSP, simultaneously. The typical chromatograms obtained for the cited drug mixtures under final optimized UPLC conditions showed retention time of 0.66, 2.02, 1.35 and 2.82 for MET, VLD, CFH and DSP, respectively; figures 2, 3.

Method validation: The proposed method was subjected to validation process to satisfy the requirements of ICH guidelines [49]. Freshly prepared stock solutions were used to establish system suitability tests. The variation in selectivity, retention time, resolution, and theoretical plates were well within the acceptable ranges for all analytes; table 1.

The drugs concentrations and peak areas were plotted to construct the calibration curves. Good linearity was established with excellent correlations (>0.999) within the concentration range of 0.5-5 µg/mL and 5-50 µg/mL for VLD and MET respectively, while covering the range of 2-20 µg/mL for both CFH and DSP. Regression parameters were computed and presented in table 2. The LOQ and LOD were determined for all the analytes (table 2); the low detection and quantification concentrations reflected the good sensitivity of the reported procedure.

The mean percentage recovery was calculated to assess the accuracy of the newly developed method. The mean recoveries were from 99.28 to 101.25 % ± 0.77-1.95 for the added drugs (table 2), representing good accuracy of the method.

Intraday and interday precision were undertaken to determine the reproducibility of the process. The % RSD values for the inter-day and intra-day measurements were ranging from 0.31-1.71%. The results listed in table 2 showed that the proposed procedure is precise.

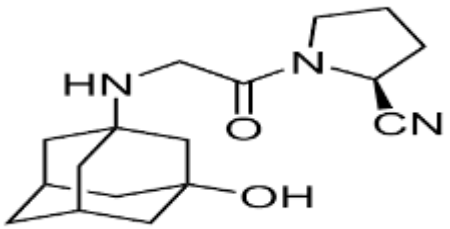
Specificity was determined by applying the proposed method to laboratory prepared mixtures containing different ratios of each of the two drugs in the two mixtures. Good recoveries for the studied drugs in both mixtures were obtained and results were presented in table 3.

The robustness of the suggested method was confirmed by performing the analysis with modifications to the flow rate of the mobile phase (±10%), mobile phase composition (±2%) and detection wavelength (±2 nm). The results showed in table 4 declares that slight modifications did not affect the resolution and tailing factor, indicating good robustness of the developed UPLC method.

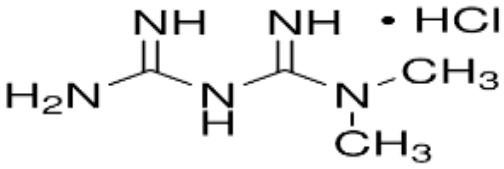
Table 5 showed statistical comparisons of the results obtained by the proposed method and reported methods [5, 44]. The calculated t- and F-values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

CONCLUSION

Ultra performance liquid chromatography (UPLC) is an innovative product that brought revolution in high performance liquid chromatography by outperforming conventional HPLC. It decreases sample run times up to a factor of 10, uses up to 95 percent less solvent and significantly improves productivity in the lab. In the present study, UPLC has been applied to the method development and validation study of binary mixtures of vildagliptin with metformin and ciprofloxacin with dexamethasone. The validation studies as per ICH guideline in accordance to linearity, accuracy, precision, LOD, LOQ and robustness proved suitability of the method for the intended use. Also, the non-interference of additives and excipients makes it suitable for determination of the studied drugs in bulk and in their combined dosage forms.



VLD



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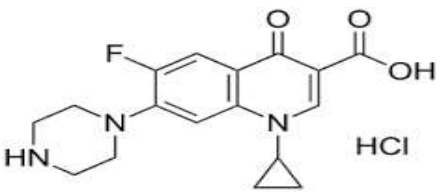
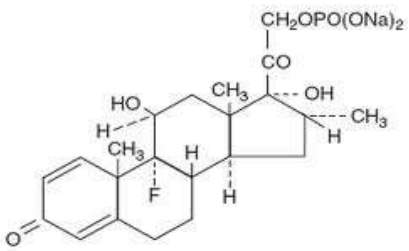


CFH



DSP

Figure1: Chemical structures of the studied drugs

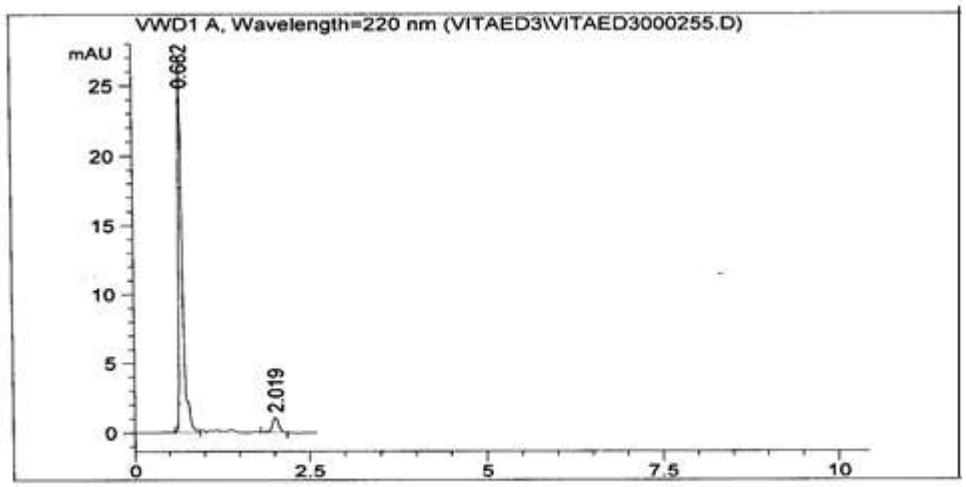


Figure 2: UPLC chromatogram of VLD and MET using a mobile phase of phosphate buffer: acetonitrile (70:30 v/v) as mobile phase

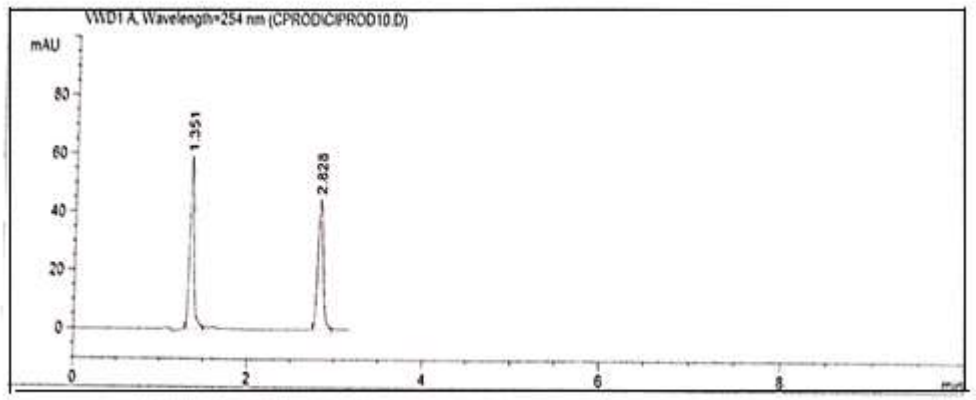


Figure 3: UPLC chromatogram of CFH and DSP using a mobile phase of phosphate buffer:acetonitrile (80:20 v/v) as mobile phase

Table 1: System suitability parameters by the proposed UPLC method

Parameter	VLD	MET	CFH	DSP
Retention time(min)	2.02	0.66	1.35	2.82
Capacity factor(k')	1.62	1.99	1.17	1.47
Resolution	10.78		15.48	
Theoretical plates	8599	7570	5973	11095

*average of 5 determinations

Table 2: Validation parameters of the proposed UPLC method for the determination of VLD, MET, CFH and DSP.

Parameter	VLD	MET	CFH	DSP
Linearity range (µg/mL)	0.5-5	5-50	2-20	2-20
LOD (µg/mL)	0.11	0.29	0.15	0.17
LOQ (µg/mL)	0.35	0.98	0.50	0.59
Regression parameters				
Slope	10.825	10.033	14.093	13.476
Intercept	-0.085	1.267	1.4714	-0.1171
Correlation coefficient (r^2)	0.9990	0.9992	0.9999	0.9999
Accuracy				
(R% of added standard ± SD)	101.25±1.02	99.98±0.77	99.28±1.95	100.39±0.97
Precision (RSD %)*				
Intraday	0.64-1.01	0.46-1.53	0.39-1.62	0.46-0.99
Interday	0.65-1.25	0.31-0.84	0.54-1.71	0.35-1.09

*average of 9 determinations

Table 3: Determination of VLD/MET and CFH/DSP in their laboratory prepared mixtures by the proposed UPLC method.

Lab - prepared mixture (Ratio VLD/MET)	Recovery % VLD	MET
1:10	100.01	100.23
1:5	99.49	99.51
1:1	99.32	100.79
Mean±S.D	99.61±0.36	100.18±0.64
Lab - prepared mixture (Ratio CFH/DSP)	**Recovery % CFH**	**DSP**
3:1	100.70	101.51
1:1	100.46	99.37
2:1	99.25	99.53
Mean±S.D	100.14±0.78	100.14±1.92

Table 4: Robustness study for the proposed UPLC method

Parameters	Changed condition	%RSD VLD	MET	CFH	DSP
Flow rate 1 mL / min	± 10%	0.921	0.762	1.095	0.679
Mobile phase ratio phosphate buffer: acetonitrile (70:30 v/v) for VLD/MET (80:20 v/v) for CFH/DSP	± 2%	0.530	0.451	0.334	0.194
λ of detection 220 nm for VLD/MET 254nm for CFH/DSP	± 2 nm	1.001	0.622	0.628	0.455

Table 5: Statistical analysis of results obtained by the proposed and reported methods [5,44] for the determination of VLD/MET and CFH/DSP in their pharmaceutical formulations.

Parameters	VLD	MET	Reported method[5]	CFH	DSP	Reported method[44]
Mean %	100.02	99.79	100.27	99.42	100.15	100.09
N	5	5	5	5	5	5
SD	0.98	1.04	1.38	1.01	0.57	1.29
Variance	0.96	1.08	1.90	1.02	0.45	0.10
t-	0.33	0.62	--	0.92	0.19	--
F-	1.98	1.76	--	1.63	3.69	--

The theoretical t- and F- values at P = 0.05 were (1.860) and (5.19) respectively.

-Reference method [5] depends on measuring ance at the wavelength of maximum absorptions of VLD and MET at 233 and 216 nm respectively, using 0.1 N NaOH as solvent.

-Reference method [44] depends on measuring the trough amplitude of the second derivative of the ratio spectra at 287.5 nm by dividing the spectra of CFH by the spectrum of 15 µg/mL DSP. Also, DSP was determined by measuring the peak amplitude of the third derivative of the ratio spectra at 254.5 nm using 10 µg /mL CFH as divisor using 0.1N NaOH as solvent.

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