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Research Article

Simultaneous HPLC determination of remdesivir and dexamethasone in the presence of metformin, sitagliptin, and glimepiride in a synthetic mixture and spiked human plasma

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Abstract

The COVID-19 pandemic has raised many questions regarding the control and therapy of type 2 diabetes and the higher risk of severe disease progression. One of the therapeutic regimens used in moderate and severe cases of COVID-19, endorsed by the World Health Organization, involves the administration of an antiviral medicinal product and a corticosteroid. The present study describes the development of a liquid chromatographic method for the simultaneous separation and quantification of Remdesivir and Dexamethasone in the presence of Metformin, Sitagliptin, and Glimepiride in a synthetic mixture. The developed method also allows determination of Remdesivir, Dexamethasone, and Glimepiride in spiked plasma samples, using Sitagliptin as internal standard. A mixture of acetonitrile and potassium dihydrogen phosphate buffer (pH 3) in a ratio of 45:55 v/v was used as a mobile phase on a C18 column. The recovery percentages from plasma ranged from 85.1 to 108.5%. The developed method can serve in routine quality control and clinical laboratory practice.

Keywords

antidiabetic drugs, bioanalysis, Covid-19, dexamethasone, remdesivir, synthetic mixture

Introduction

With its emergence and rapid spread, COVID-19 has fundamentally changed people's lives worldwide. The consequences were serious – health problems (World Health Organization 2020) and also social and economic ones (Pak et al. 2020; Smit et al. 2023). Because of the deadly nature of the disease, researchers' efforts have focused both on vaccine development and on the use and retargeting of appropriate drugs for prevention and treatment. Among these drugs, Remdesivir (RDV) and Dexamethasone (DXM) seem to have gained the most popularity. Their use, alone or in combination for moderate and severe cases of hospitalized patients with COVID-19, was approved by the World Health Organization (Spinner et al. 2020; Lin et al. 2021; Morris et al. 2021; Qureshi et al. 2021; Wong et al. 2022; National Institutes of Health 2023). To date, despite the containment of the infection and its

impact, COVID-19 remains a major threat (World Health Organization 2023), especially in patients with chronic diseases. Accumulated epidemiological evidence indicates that diabetes and cardiovascular disease are major risk factors for the development of severe COVID-19 infection (Abu-Farha et al. 2020; Chakraborty et al. 2020; Liu et al. 2020; Muniangi-Muhitu et al. 2020; Peric and Stulnig 2020; Tadic et al. 2020; Wallia et al. 2020; Czupryniak et al. 2021; Fleming et al. 2021; Landstra and de Koning 2021) and increased mortality (Kumar et al. 2020; Dennis et al. 2021; Varikasuvu et al. 2021; Wu et al. 2021). Diabetes mellitus is one of the most significant diseases in the world and shows a tendency towards a constant increase in the number of patients (Saeedi et al. 2019). Diabetic patients have been found to have increased rates of severe disease and mortality during COVID-19 infection compared to non-diabetic individuals (Akbariqomi et al. 2020; Conway et al. 2020; Maddaloni et al. 2020; Czupryniak et al. 2021; Khunti et al. 2021). The specificity and individual approach in antidiabetic therapy and its combination with antiviral and/or corticosteroid antiviral therapy requires strict control, both in terms of the quality of the administered medications and in terms of monitoring their plasma concentrations. High-performance liquid chromatography (HPLC) is a versatile technique, achieving good resolution, high specificity, and sensitivity, and is the method of choice for the analysis of multicomponent mixtures and complex samples. In the literature, there are various publications on developed HPLC methods for the analysis of both RDV and DXM, as well as antidiabetic drugs alone (Garcia et al. 2003; Song et al. 2004; Rabbaa-Khabbaz et al. 2005; Kumar et al. 2006; Kar and Choudhury 2009; Dubala et al. 2012; Chhetri et al. 2013, 2014; Mohd et al. 2014; Iqbal et al. 2018; Sultana et al. 2018; Alvarez et al. 2020; Ibrahim et al. 2021; Jitta et al. 2021; Kishore et al. 2021; Samad et al. 2023), in combination (Jain et al. 2008; Bhende et al. 2012; El-Enany et al. 2012; Ramesh and Habibuddin 2014; Priya et al. 2016; Al Bratty et al. 2017; Kavitha et al. 2017; Sirigiri et al. 2018; Sebaiy et al. 2019, 2020; Sharma et al. 2019; Shakoor et al. 2020; Emam et al. 2022), in dosage forms (Garcia et al. 2003; Jain et al. 2008; Kar and Choudhury 2009; Bhende et al. 2012; El-Enany et al. 2012; Chhetri et al. 2013; Mohd et al. 2014; Ramesh and Habibuddin 2014; Priya et al. 2016; Kavitha et al. 2017; Sirigiri et al. 2018; Sultana et al. 2018; Sebaiy et al. 2019; Sharma et al. 2019; Shakoor et al. 2020; Ibrahim et al. 2021; Jitta et al. 2021; Samad et al. 2023) and in biological matrices (Song et al. 2004; Rabbaa-Khabbaz et al. 2005; Kumar et al. 2006; Dubala et al. 2012; El-Enany et al. 2012; Chhetri et al. 2014; Al Bratty et al. 2017; Iqbal et al. 2018; Alvarez et al. 2020; Sebaiy et al. 2020; Shakoor et al. 2020; Kishore et al. 2021; Emam et al. 2022), but (to the best of our knowledge) no method has been developed and validated for the simultaneous quantification of antidiabetic, antiviral and corticosteroid agents in a synthetic mixture or biological matrix. Thus, the aim of the present study is to make an attempt to develop an HPLC method for the simultaneous analysis of RDV and DXM in the presence of some antidiabetic medicinal substances, such as Metformin (MTF), Sitagliptin (STG) and Glimepiride (GLM) in a synthetic mixture, as well as to use the method for the analysis of spiked plasma samples. The chemical structures of the studied drugs are presented in Fig. 1.

Materials and methods

Chemicals and reagents

All chemicals and reagents used for method development were HPLC grade. The analytical standards of RDV, DXM, MTF, STG, and GLM were purchased from Sigma-Aldrich

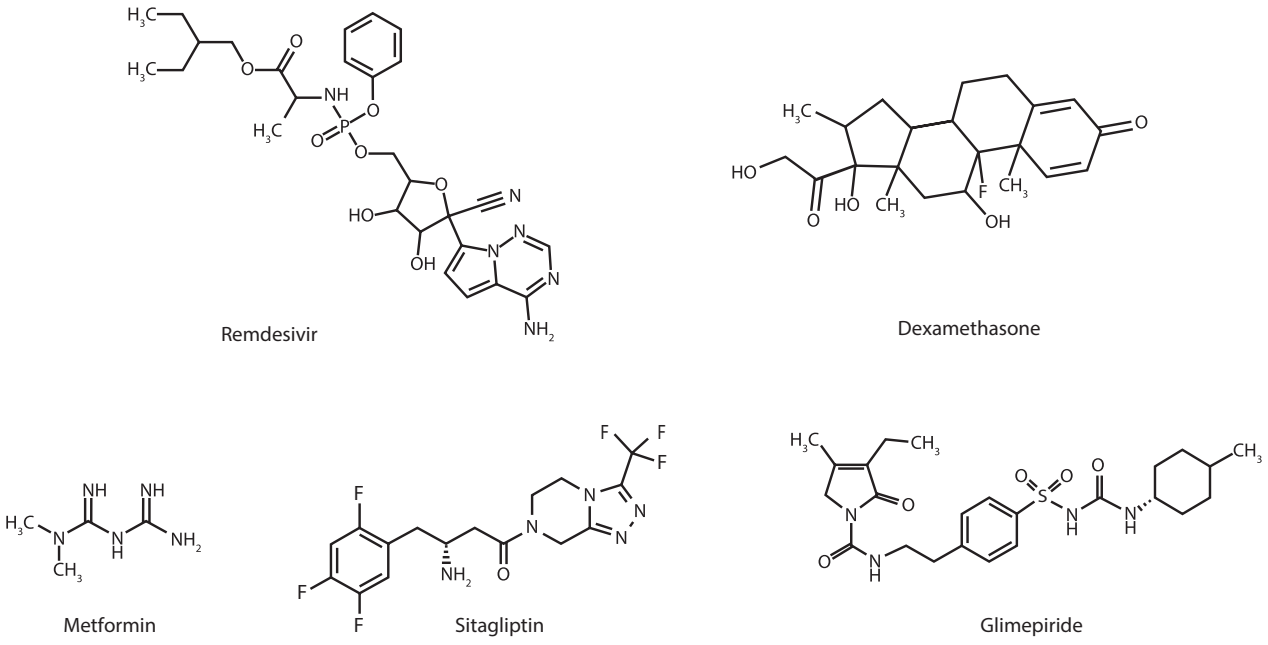

Figure 1. Chemical structures of RDV, DXM, MTF, STG and GLM.

Co. Acetonitrile, methanol, and potassium dihydrogen phosphate used for mobile phase and stock solutions preparation were of HPLC grade. A blank human plasma standard (Sigma-Aldrich Co.) was used for calibration curve construction. All additional reagents needed to develop the analytical method were suitable for HPLC analysis.

Preparation of stock solutions and mixed stock solutions

The stock solutions of RDV, DXM, MTF, STG (1 mg/ml), and GLM (0.2 mg/ml) were prepared by dissolving the required amount of each substance in methanol and diluting it to 20.0 ml with the same solvent. A mixed stock solution (solution A, 40 μg/ml) was prepared by transferring suitable aliquots of each stock solution to one and the same 50.0 ml volumetric flask and further diluted with methanol. For plasma studies stock solutions of RDV, DXM (4 mg/ml), and GLM (0.2 mg/ml) were prepared in methanol. A mixed stock solution containing RDV, DXM, and GLM (solution B, 160 μg/ml) was prepared by transferring the required aliquots of the RDV, DXM, and GLM stock solutions to a 20.0 ml volumetric flask and further diluted with methanol.

Preparation of working solutions

Six working solutions (RDMSG -SM1, RDMSG -SM2, RDMSG -SM3, RDMSG -SM4, RDMSG -SM5 and RDMSG -SM6) in the concentration range of 1–24 μg/ml and three working quality control solutions (RDMSG -QC1, RDMSG -QC2, and RDMSG -QC3) with concentrations 1.6, 6, and 20 μg/ml were prepared by appropriately diluting aliquots from the solution A with methanol. For plasma studies six working solutions (RDG-P1, RDG-P2, RDG-P3, RDG-P4, RDG-P5, and RDG-P6) in the concentration range of 5–120 μg/ml and three working quality control solutions (RDG-QC1, RDG-QC2, and RDG-QC3) with concentrations 8, 30, and 100 μg/ml were prepared by appropriately diluting aliquots from the solution B with methanol. All solutions were stored at 2–4 °C temperature before analysis.

Preparation of internal standard solution

For plasma analysis, STG was chosen as an internal standard (IS). The solution was prepared by dissolving 40 mg of the substance in methanol and diluting it to 50.0 ml with the same solvent. The obtained concentration was 0.8 mg/ml.

Preparation of calibration and quality control solutions for the plasma assay

Calibration and quality control solutions were prepared in standard human plasma. To 400 μl blank plasma a 100 μl of RDG-P1, RDG-P2, RDG-P3, RDG-P4, RDG-P5, RDG-P6, RDG-QC1, RDG-QC2 and RDG-QC3 were added, respectively. The resulting samples were vortexed for 2 min. The obtained concentrations were 1, 2, 4, 8, 16, and 24 μg/ml for the calibration standards and 1.6, 6, and 20 μg/ml for the quality control samples.

Sample preparation

Plasma protein precipitation was performed according to the previously published method (Smerikarova et al. 2023) with some modifications and adjustments. A plasma aliquot of 150 μl was mixed with 50 μl of the IS solution, then the sample was vortexed for 5 min. Further on, 600 μl of acetonitrile was added as a precipitating agent. After homogenization on a vortex mixer for 10 min, 15 min of sonication in an ultrasonic bath and 15 minutes of shaking at 500 rpm were applied. The supernatant was separated after 10 min of centrifugation at 13 000 rpm and 600 μl were filtered through a 0.45 μm syringe filter.

Chromatographic conditions

A SHIMADZU Corporation chromatographic system consisting of online degassing unit DGU-20A5, solvent delivery unit LC-20AD, autosampler Sil-20A, column oven CTO-20A, and UV-VIS detector SPD-20A was. The recording and processing of the results were done with Lab Solution Software. An RP-18 (250 × 4.6 mm, 5 μm) chromatographic column, equipped with a suitable guard column, was applied as a stationary phase. Isocratic elution was performed at a flow rate of 1.0 ml/min, and at ambient temperature. A mixture of acetonitrile and potassium dihydrogen phosphate buffer (pH 3) in a ratio of 45:55 v/v was used as a mobile phase. The mobile phase was filtrated and degassed in an ultrasonic bath before use. UV – VIS detector was set at 250 nm and the injection volume was 20 μl.

Results and discussion

Method development and optimization

During the development of the chromatographic method, columns with different hydrocarbon chain lengths were tested, namely – octadecylsilane (C18) and octylsilane (C8). Considering the very short MTF retention time on the C8 column as well as the better separation on the C18 column, the C18 column was chosen for further studies. The effect of column length was investigated (two columns of different lengths -150 × 4.6 mm and 250 × 4.6 mm were used) and the longer column was preferred as it showed some additional increase in MTF retention time.

To find a suitable mobile phase, experiments were carried out with different compositions and ratios of solutions. Acetonitrile was preferred as the organic solvent because the use of methanol resulted in broad, diffuse, and asymmetric peaks. However, the application of mixtures of acetonitrile and water in different ratios did not produce the desired result. As the percentage of acetonitrile

increased, overlapping peaks were observed and conversely, as the acetonitrile content decreased, the peaks broadened and along with this, the retention time of the last eluting peak increased too much. The introduction of a phosphate buffer in the composition of the mobile phase led to an improvement in the results. The effect of pH in the range of 3 to 6 was investigated, with the best results obtained at pH 3. The effect of buffer concentration (concentrations from 0.5 mM to 20 mM) on the separation and peak shape was also investigated. A concentration of 20 mM was the most suitable. It should be noted that lowering the concentration of the buffer solution results in broader peaks especially for antidiabetic agents and also to an unacceptable increase in the retention time of the latter component. Finally, a mixture of acetonitrile and potassium dihydrogen phosphate buffer (pH 3) in a ratio of 45:55 v/v was used as a mobile phase. Varying the flow rate and temperature did not significantly improve the separation of the compounds, so 1 ml/min flow rate and room temperature were chosen. The absorption maxima of the investigated compounds vary in relatively wide ranges, approximately from 230 nm for GLM to 270 nm for STG. Therefore, considering the spectra of the compounds (Fig. 2), as a good compromise, the UV detection was performed at 250 nm.

Method validation

The developed method was validated according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (International Council for Harmonisation 2022) by various parameters including selectivity, linearity, accuracy, precision, system suitability, limit of detection and limit of quantification.

Selectivity

A method is considered selective when it can distinguish the target compounds from other components in the sample. The selectivity of the proposed method was proven by its capability to determine RDV, DXM, MTF, STG, and GLM in their synthetic mixture without interference. As can be seen from the representative chromatograms (provided in Fig. 3A, B) there are no interferences from mobile phase components during the analysis of RDV, DXM, MTF, STG, and GLM in their synthetic mixture. The peaks of all five analyzed drug substances are well separated.

System suitability tests

System suitability tests were performed in order to evaluate the performance of the chromatographic system. Parameters like retention time, column efficiency (column theoretical plates), capacity factor, selectivity, resolution, and tailing factor were determined by a sixfold analysis of the test sample at the concentration of 8 μg/ml. The requirements of the European Pharmacopoeia were fulfilled, as can be seen from the detailed results, expressed as mean values in Table 1.

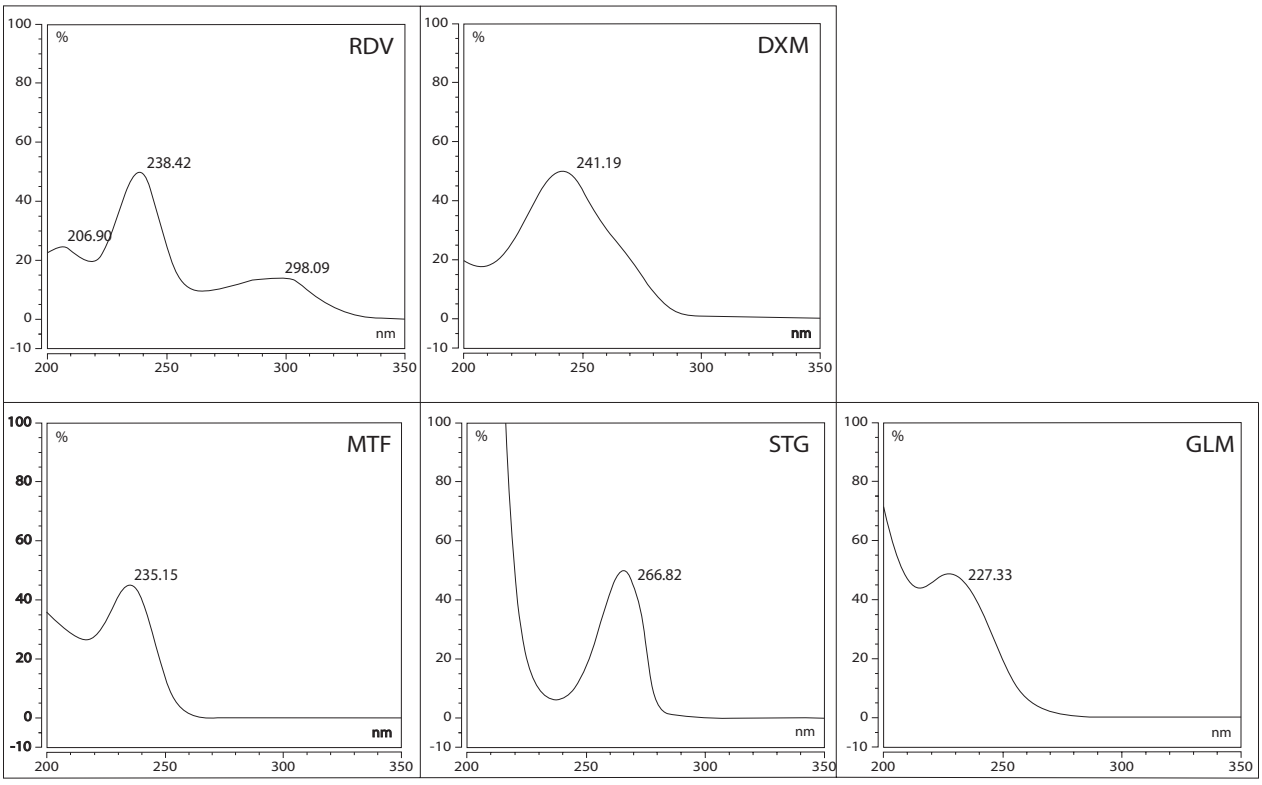

Figure 2. UV spectra of RDV, DXM, MTF, STG and GLM.

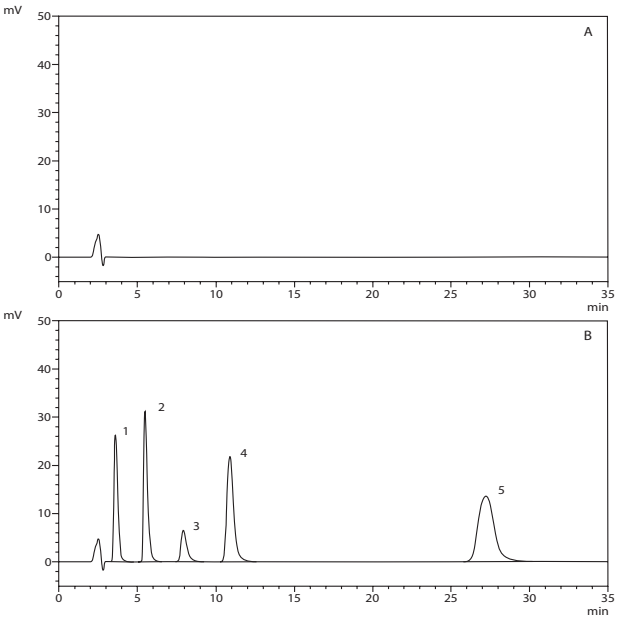

Figure 3. Representative chromatograms obtained from **A.** The mobile phase and **B.** Synthetic mixture. **Legend:** Peak numbers: MTF-1, DXM-2, STG-3, RDV-4, and GLM-5.

Table 1. Results of the system suitability tests.

Parameter	Compound				
(Acceptance criteria, Ph. Eur.)	MTF	DXM	STG	RDV	GLM
t_R, min	3.65	5.55	7.98	10.91	27.21
N (NLT 2000)	2006	2282	2180	3021	3576
κ' (NLT 2.0)	2.10	2.19	2.15	3.31	9.75
α (NLT 1.0)	1.62	2.70	1.81	1.54	2.95
R_S (NLT 2.0)	2.08	3.94	4.24	3.96	12.47
T_f (NMT 2.0)	1.29	1.47	1.56	1.37	1.36

t_R: retention time; N: theoretical plates; κ': capacity factor; α: selectivity; R_S: resolution; T_f: tailing factor; Ph. Eur.: European Pharmacopoeia; NLT: no less than; NMT: no more than.

Linearity and range

Linearity studies were performed to track the dependence of the analytical signal on the concentration of the analytes in the samples. Six standard solutions containing the analyzed substances were used in a concentration range from 1 to 24 μg/ml. For calibration curve construction, peak areas against solution concentrations were plotted. The achieved high correlation coefficients (R2>0.999) showed good linearity of the proposed method. Detailed results are listed in Table 2.

Accuracy and precision

Quality control samples at three concentration levels within the range of the calibration curve were used for accuracy and precision studies. Three independently prepared samples were triplicate analyzed in one day or on three consecutive days, to assess the accuracy and precision within a run and between runs. Reproducibility and accuracy were assessed by a coefficient of variance (CV%) and by the percentage deviation of the average concentration compared to the weighted one (d%). The coefficients of variance were below 2.5% and d% ranged from -3.5 to 6.2%. Detailed results are listed in Table 3.

Table 2. Linearity, accuracy and precision results for the RDV, DXM, MTF, GLM and STG calibration curves.

Compound	Concentration (μg/ml)	Mean ± SD	CV%	d%
RDV	1	0.928 ± 0.015	1.634	-7.243
	2	2.018 ± 0.008	0.400	0.897
	4	4.190 ± 0.002	0.049	4.752
	8	8.001 ± 0.036	0.447	0.013
	16	15.969 ± 0.023	0.147	-0.193
	24	23.994 ± 0.017	0.069	-0.024
	Linear equation	y = 41594x + 2187.3		
	R^2	0.9998		
DXM	1	0.977 ± 0.006	0.631	-2.324
	2	2.028 ± 0.007	0.331	1.397
	4	4.073 ± 0.025	0.623	1.826
	8	8.056 ± 0.042	0.519	0.696
	16	15.983 ± 0.042	0.266	-0.105
	24	23.983 ± 0.030	0.124	-0.071
	Linear equation	y = 34037x + 426.62		
	R^2	0.9999		
MTF	1	0.934± 0.019	2.041	-6,551
	2	2.119± 0.049	2.295	5.941
	4	4.097 ± 0.079	1.918	2.414
	8	8.218 ± 0.077	0.939	2.727
	16	16.047 ± 0.067	0.419	0.294
	24	23.886 ± 0.052	0.218	-0.477
	Linear equation	y = 30082x + 18279		
	R^2	0.9995		
GLM	1	1.009 ± 0.077	7.595	0.907
	2	1.968 ± 0.033	1.694	-1.616
	4	3.987 ± 0.076	1.908	-0.317
	8	7.895 ± 0.060	0.762	-1.309
	16	16.304 ± 0.007	0.041	1.902
	24	23.837 ± 0.152	0.640	-0.680
	Linear equation	y = 20722x – 462.82		
	R^2	0.9997		
STG	1	0.929 ± 0.049	5.298	-7.116
	2	2.014 ± 0.099	4.904	0.718
	4	4.272 ± 0.039	0.923	6.807
	8	8.028 ± 0.137	1.708	0.347
	16	15.997 ± 0.085	0.533	-0.022
	24	23.961 ± 0.311	1.299	-0.161
	Linear equation	y = 1649.7x – 191.77		
	R^2	0.9999		

SD: standard deviation; CV: coefficient of variation.

Limit of detection (LOD) and limit of quantification (LOQ)

Limits of detection and limits of quantification were determined experimentally by the signal-to-noise ratio. The LODs achieved were 0.05 μg/ml for MTF, DXM, and RDV. For STG LOD was 0.5 μg/ml and for GLM 0.3 μg/ml. The limits of quantification were 0.2 μg/ml for MTF, DXM, and RDV, and 1.0 μg/ml for STG and GLM.

Plasma samples

A protein precipitation procedure was used as a simple and fast sample preparation method. Unfortunately, under specific chromatographic conditions, MTF cannot be

Table 3. Accuracy, reliability and reproducibility in a run and in time (n=3).

Compound	Concentration (μg/ml)	Intraday			Interday		
		Mean ± SD	CV%	d%	Mean ± SD	CV%	d%
RDV	1.6	1.604 ± 0.006	0.402	0.240	1.580 ± 0.009	0.546	-1.221
	6	5.988 ± 0.027	0.448	-0.207	6.205 ± 0.029	0.466	3.411
	20	19.975 ± 0.029	0.147	-0.127	20.188 ± 0.089	0.440	0.938
DXM	1.6	1.620 ± 0.005	0.332	1.241	1.609 ± 0.005	0.284	0.585
	6	6.039 ± 0.031	0.519	0.644	6.206 ± 0.025	0.396	3.441
	20	19.982 ± 0.053	0.266	-0.089	20.110 ± 0.065	0.323	0.551
MTF	1.6	1.574 ± 0.039	2.472	-1.654	1.574 ± 0.020	1.291	-1.640
	6	6.261 ± 0.058	0.924	4.350	6.369 ± 0.064	1.005	6.154
	20	20.211 ± 0.084	0.415	1.053	20.517 ± 0.103	0.500	2.587
GLM	1.6	1.570 ± 0.005	0.306	-1.895	1.631 ± 0.010	0.635	1.961
	6	5.872 ± 0.054	0.912	-2.139	5.788 ± 0.077	1.332	-3.537
	20	19.881 ± 0.089	0.448	-0.595	20.136 ± 0.026	0.130	0.682
STG	1.6	1.590 ± 0.006	0.387	-0.611	1.587 ± 0.005	0.291	-0.794
	6	5.855 ± 0.047	0.808	-2.421	6.008 ± 0.036	0.595	0.139
	20	20.240 ± 0.018	0.090	1.198	20.450 ± 0.100	0.490	2.250

SD: standard deviation; CV: coefficient of variation.

determined in plasma samples due to its very short retention time. An overlap of the plasma and MTF peaks was observed. However, the proposed method allows the simultaneous determination of RDV and DXM in the presence of the GLM and IS. All drug substances were well separated and no interferences from the biological matrix components were observed (Fig. 4A, B).

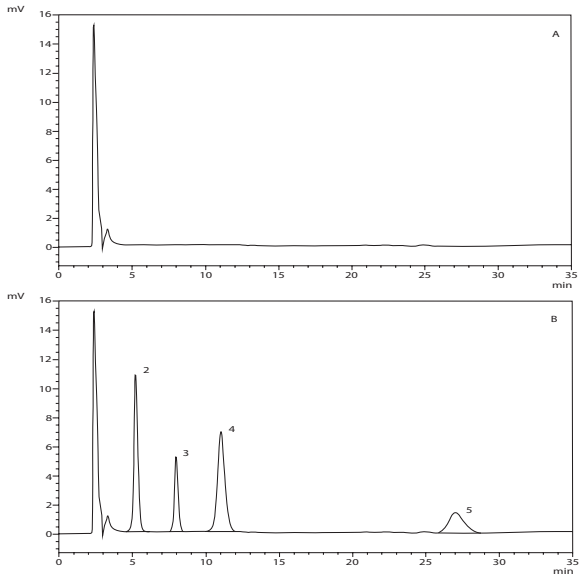

Figure 4. Representative chromatograms obtained from **A.** The blank and **B.** Spiked plasma samples. **Legend:** Peak numbers: DXM-2, STG-3, RDV-4 and GLM-5.

The area ratio of the chromatographic peaks of each analyte and the IS was plotted against concentration for the calibration curve in plasma. Linear relationship over the studied concentration range (from 1 to 24 μg/ml) and satisfactory correlation coefficients (R2>0.997) were observed. The details are summarized in Table 4.

Three QC samples at different concentration levels were analyzed for within-run and between-run accuracy and precision studies. The coefficient of variance (CV%) and the percentage deviation of the average concentration compared to the weighted one (d%) were used for reproducibility and accuracy assessment. The coefficients of variance were below 3.7% and d% ranged from -7.3 to 8.5%. The results summarized in Table 5 confirmed the method as accurate and reproducible.

Table 4. Linearity, accuracy and precision results for the RDV, DXM and GLM calibration curves in plasma.

Compound	Concentration (μg/ml)	Mean ± SD	CV%	d%
RDV	1	0.894 ± 0.004	0.403	-10.600
	2	1.838 ± 0.026	1.390	-8.117
	4	3.623 ± 0.022	0.612	-9.417
	8	8.022 ± 0.023	0.280	0.279
	16	14.698 ± 0.070	0.478	-8.140
	24	23.898 ± 0.315	1.320	-0.424
	Linear equation	y = 0.1142x – 0.0444		
	R^2	0.9971		
DXM	1	0.891 ± 0.007	0.730	-10.867
	2	1.799 ± 0.021	1.189	-10.067
	4	3.556 ± 0.038	1.074	-11.108
	8	8.018 ± 0.019	0.232	0.229
	16	15.035 ± 0.057	0.382	-6.033
	24	24.614 ± 0.126	0.513	2.557
	Linear equation	y = 0.0974x – 0.0348		
	R^2	0.9972		
GLM	1	1.062 ± 0.029	2.745	6.167
	2	2.095 ± 0.190	9.081	4.733
	4	3.952 ± 0.068	1.712	-1.200
	8	7.968 ± 0.048	0.598	-0.396
	16	15.122 ± 0.936	6.191	-5.490
	24	24.669 ± 0.107	0.433	2.787
	Linear equation	y = 0.0395x – 0.0035		
	R^2	0.9971		

SD: standard deviation; CV: coefficient of variation.

The recovery studies were performed by blank plasma samples which were spiked with a laboratory-prepared mixture (RVD, DXM, and GLM) and IS at three different concentration levels. The obtained recovery percentages, ranging from 85.1% to 108.5% are listed in Table 6.

Comparison with published methods

Table 7 presents comparative data for the proposed method and some previously published reports. It can be seen that in terms of limits of detection and quan-

Table 5. Accuracy, reliability and reproducibility in a run and in time (n=3).

Compound	Spiked concentration (µg/ml)	Intraday			Interday		
		Mean ± SD	CV%	d%	Mean ± SD	CV%	d%
RDV	1.6	1.544 ± 0.031	1.980	-3.521	1.533 ± 0.046	3.016	-4.208
	6	5.806 ± 0.147	2.528	-3.228	5.764 ± 0.144	2.501	-3.933
	20	18.543 ± 0.145	0.781	-7.283	18.600 ± 0.116	0.621	-7.002
DXM	1.6	1.565 ± 0.006	0.399	-2.188	1.575 ± 0.015	0.940	-1.563
	6	6.165 ± 0.159	2.575	2.756	6.013 ± 0.140	2.331	0.217
	20	19.085 ± 0.048	0.253	-4.575	19.084 ± 0.064	0.334	-4.578
GLM	1.6	1.611 ± 0.005	0.287	0.667	1.568 ± 0.014	0.905	-2.021
	6	6.509 ± 0.141	2.169	8.483	6.318 ± 0.228	3.612	5.294
	20	20.549 ± 0.046	0.223	2.747	20.603 ± 0.178	0.865	3.017

SD: standard deviation; CV: coefficient of variation.

Table 6. Recoveries of RVD, DXM and GLM from spiked human plasma (n=3).

Compound	Spiked concentration (µg/ml)	Concentration found (µg/ml)	Recovery (%) (mean ± SD)	CV %
RDV	1.6	1.37	85.38 ± 3.19	3.74
	6	5.75	95.79 ± 2.43	2.53
	20	18.60	93.00 ± 0.58	0.62
DXM	1.6	1.36	85.08 ± 0.51	0.60
	6	6.01	100.22 ± 2.34	2.33
	20	19.08	95.42 ± 0.32	0.33
GLM	1.6	1.61	100.66 ± 0.30	0.29
	6	6.51	108.49 ± 2.35	2.17
	20	20.55	102.75 ± 0.23	0.23

SD: standard deviation; CV: coefficient of variation.

Table 7. Comparison between the proposed method and some previously published reports.

Compound	t_R (min)	Linearity (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Reference [№]
RDV	10.91	1–24	0.05	0.2	Proposed method
DXM	5.55	1–24	0.05	0.2	
MTF	3.65	1–24	0.05	0.2	
GLM	27.21	3–72	0.3	1.0	
STG	7.98	3–72	0.5	1.0	
Determination of studied compounds in bulk, mixtures and formulations					
RDV	6	0.025–2.5	1.95×10^{-3}	6.49×10^{-3}	(Jitta et al. 2021)
RDV	16	5–50	0.9	2.76	(Samad et al. 2023)
RDV	8.5	5–100	0.5	2.00	(Ibrahim et al. 2021)
DXM	8.5	5–60	0.47	1.41	(Garcia et al. 2003)
MTF	2.66	10–5000	-	-	(Jain et al. 2008)
GLM	10.17	1–10	-	-	
MTF	3.06	5–100	0.05	0.17	(Sebaiy et al. 2019)
GLM	4.33	5–100	1.2	4.0	
MTF	2.57	20–200	-	-	(Ramesh and Habibuddin 2014)
GLM	9.39	10–150	-	-	
MTF	3.70	25–250	2.92	8.72	(Priya et al. 2016)
GLM	4.47	25–250	2.92	8.72	
MTF	2.9	20–80	0.6	2.0	(Kavitha et al. 2017)
STG	3.9	20–80	0.4	1.2	
MTF	2.4	50–150	-	-	(Sirigiri et al. 2018)
STG	17.0	50–150	-	-	
MTF	2.11	10–80	0.4	1.4	(Sharma et al. 2019)
STG	5.30	1–8	0.08	0.28	
MTF	2.4	50–150	0.03	10.3	(Bhende et al. 2012)
STG	3.01	50–150	0.03	10.1	
Determination of studied compounds in biological matrices					
RDV	9.1	0.01–0.07	-	0.01	(Kishore et al. 2021)
RDV	2.4	1–5000	0.5	1.0	(Alvarez et al. 2020)
DXM	-	-	-	10	(Song et al. 2004)
DXM	9.9	0.25–6.0	-	-	(Kumar et al. 2006)
RDV	3.65	0.1–10	0.1	10	(Emam et al. 2022)
DXM	3.08	0.1–10	0.1	10	
MTF	9.93	0.125–2.5	0.062	0.125	(Chhetri et al. 2014)
MTF	3.36	10–140	2.0	6.0	(Shakoor et al. 2020)
GLM	-	0.01–1	-	0.01	(Rabbaa-Khabbaz et al. 2005)
GLM	4.66	0.125–12.5	0.04	0.1	(El-Enani et al. 2012)
STG	6.1	0.01–1	1×10^{-3}	0.01	(Dubala et al. 2012)
STG	6.7	0.12–31	-	0.12	(Iqbal et al. 2018)
MTF	1.24	2.5–100	0.05	-	(Sebaiy et al. 2020)
GLM	2.77	2.5–100	0.1	-	
MTF	-	0.5×10^{-3}-	0.17×10^{-3}	-	(Al Bratty et al. 2017)
STG	-	0.4	1.76×10^{-3}	-	
		0.01–0.5			

tification, as well as linearity, the results obtained are better in some cases. It can be said that the obtained retention time results occupy an intermediate position compared to the published ones. An exception is the retention time of GLM, which is higher than the published ones.

Regarding the recovery rate (Table 6) for RDV, DXM, and GLM from the biological samples, the obtained

results can be considered similar to the published reports. In the available literature, the recovery rates for RDV range from 97% to 103% (Kishore et al. 2021; Emam et al. 2022), for DXM from (77%–82%) (Kumar et al. 2006) to (97%–103%) (Emam et al. 2022), and for GLM from 80% to 98%) (El-Enany 2012; Sebaiy et al. 2020).

However, the obtained results can hardly be compared due to the lack of published methods dealing with the simultaneous separation and quantification of the studied medicinal substances.

Conclusion

A simple and accurate liquid chromatographic method was developed and validated for the simultaneous determination of RDV and DXM in the presence of MTF, STG, and GLM in their laboratory-prepared mixture. The proposed method allows separation and quantification of RDV, DXM, and GLM in biological matrix. The method offers simple sample preparation and a recovery rate of 85% to 108% for the biological matrix. Despite some limitations and drawbacks, the proposed method provides opportunities to expand research on the simultaneous separation and determination of RDV, DXM, and other antidiabetic drugs, in routine quality control and clinical laboratory practice.

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