ASSESSMENT OF CHROMATOGRAPHIC SEPARATIONS FOR ANTIHYPERTENSIVE AND ANTIDIABETIC DRUGS

SOWMYALAKSHMI VENKATARAMAN1*, HARITHA G2

1Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. 2Research Scholar, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. Email: sowmyamahesh30@gmail.com

ABSTRACT

Context: The focus of this review is to compile the different chromatographic methods that were reported earlier for the analysis of different antihypertensive and antidiabetic drugs.

Objective: The magnitude of chemical entities investigated and entering into the medicinal field for various health-related ailments is escalating year after year. The drugs are either innovative entities or fractional structural variation of the preexisting chemical molecule. These drugs may exhibit unexpected toxicities after Phase IV of clinical trials, resulting in their withdrawal from the market. Under these circumstances, analytical measures for these drugs may not be accessible in the pharmacopeias. The main aim of this work is to compile the different analytical techniques for the quantification of various antihypertensive drugs and antidiabetic drugs.

Methods: The present work is to thoroughly study the literature for the application of different analytical techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy/tandem mass spectroscopy (LC-MS/MS) for the quantification of antihypertensive drugs and anti-diabetic drugs.

Results: The present study attempts to collate various analytical techniques that were developed and validated for the estimation of few important antidiabetic and antihypertensive drugs either in pure, individually or combined with other pharmaceutical dosage form by HPLC, LC-MS/MS, and high-performance thin-layer chromatography techniques.

Conclusion: Different chromatographic methods are considered to be rapid tools for qualitative and quantitative analysis of newer chemical entities in pharmaceuticals. The amount of these newer chemical entities which are reaching the pharmaceutical market is increasing day by day nevertheless there exists a lag in establishing the standard protocols for the identification, impurity profiling, related substance and assay method. Hence, the present review compiles the different analytical methods that were reported in the literature and thus helps the researchers and chemists to make use of the analytical techniques for the quantification and validation of various antidiabetic and antihypertensive drugs.

Keywords: Liquid chromatography, High-performance liquid chromatography, Antihypertensive, Antidiabetic, Oral hypoglycemics, Liquid chromatography-mass spectroscopy/tandem mass spectroscopy.

INTRODUCTION

Nowadays, hypertension is prevalent among individuals across the world and is an important modifiable risk factor for various cardiovascular ailments. It ultimately affects the functioning of the heart, kidney, and brain. Antihypertensive drugs comprise vasodilators, diuretics, angiotensin receptor blockers, and sympathoplegic agents [1]. Vasodilators reduce the tension in the vascular muscle; a diuretic depletes the levels of sodium and lessens the volume of blood; angiotensin receptor blockers hinder the angiotensin-converting enzyme; and sympathoplegic agents taper off the cardiac output [2].

Elevated levels of glucose in blood and fluctuations in the levels of insulin production by the pancreas provoke the disease called diabetes mellitus. Other than insulins, oral hypoglycemics such as antidiabetic drugs comprise thiazolidinediones, biguanides, incretin-based drugs, sulfonylureas, alpha-glucosidase inhibitors, glinides and amylin analog. All these drugs help to regulate the amount of insulin in the blood [3].

The important objective of this study is to collate the different bioanalytical techniques that are available for various drugs in the class of antihypertensives and antidiabetics. Accordingly, a thorough literature search from the PUBMED (NCBI) database using appropriate keywords was performed. Table 1 summarizes the salient features of different antihypertensive and antidiabetic drugs. The present review compiles the reported bioanalytical methods with more emphasis for approved drugs such as pioglitazone, metformin, glipizide, amlodipine, nifedipine, valsartan, and captopril.

Hence, the present manuscript is based on the various analytical method developments and validation carried out for antihypertensive and antidiabetic drugs. In this review, a detailed study on the assay, impurity profiling, and stability indication assay, bio-analytical methods that were carried out by various chromatographic methods are discussed for hypertension and diabetes drugs in their pure form, individually or in combined pharmaceutical dosage form.

Analysis involving liquid chromatography-mass spectroscopy/tandem mass spectroscopy (LC-MS/MS)

Pioglitazone and its metabolic products (III and IV) were analyzed in the plasma using LC-MS/MS involving electrospray tandem mass spectrometer system. The drug was extracted from plasma using liquid-liquid extraction (LLE) with methyl t-butyl ether-a-butyl chloride...
### Table 1: Salient features of some antihypertensive and antidiabetic drugs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade name</td>
<td>Glipizide</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{18}H_{18}NO_{5}</td>
</tr>
<tr>
<td>CAS number</td>
<td>52-42-8</td>
</tr>
<tr>
<td>pKa</td>
<td>6.5</td>
</tr>
<tr>
<td>Majorly metabolized by CYP2C9</td>
<td></td>
</tr>
<tr>
<td>Protein binding</td>
<td>98–97%</td>
</tr>
<tr>
<td>Route of elimination</td>
<td>Urine</td>
</tr>
<tr>
<td>Oral bioavailability</td>
<td>≤3–5 h</td>
</tr>
<tr>
<td>Half-life</td>
<td>5–7 h</td>
</tr>
</tbody>
</table>

#### Antidiabetic drugs

<table>
<thead>
<tr>
<th>Antidiabetic drugs</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glimepiride</td>
<td>Molecular formula: C_{18}H_{18}NO_{5}</td>
</tr>
<tr>
<td>Glipizide</td>
<td>Molecular formula: C_{18}H_{18}NO_{5}</td>
</tr>
<tr>
<td>Metformin</td>
<td>Molecular formula: C_{7}H_{13}NO_{6}</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>Molecular formula: C_{18}H_{18}NO_{5}</td>
</tr>
</tbody>
</table>

#### Antihypertensive drugs

<table>
<thead>
<tr>
<th>Antihypertensive drugs</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>Molecular formula: C_{21}H_{27}NO_{3}</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Molecular formula: C_{21}H_{27}NO_{3}</td>
</tr>
<tr>
<td>Captopril</td>
<td>Molecular formula: C_{12}H_{18}O_{7}</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Molecular formula: C_{21}H_{27}NO_{3}</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Molecular formula: C_{21}H_{27}NO_{3}</td>
</tr>
</tbody>
</table>

#### LC-MS study

LC-MS study was carried out using human plasma for the determination of metformin, atorvastatin, and glimepiride [8]. The extraction was done with protein precipitation with acetonitrile. Pharmacokinetic parameters were investigated in nifedipine, montelukast, and gliclazide, and they were analyzed using SB-C_{18} with formic acid (0.1%) 45:55 acetonitrile as mobile phase. Analysis of sacubitril and valsartan in rat plasma was carried out using liquid chromatography, and further, the eluent was subjected for quantitative by ESI in positive mode. The study was carried out using Hypersil GOLD C_{18} column, with acetonitrile and formic acid (0.1%) as mobile phase. Before the analysis, the drugs were extracted using ultrasonic sonication. 

### Analysis using high-performance liquid chromatography (HPLC)

Telmisartan and pioglitazone were simultaneously separated using ODS-3v column with 65% of ammonium dihydrogen phosphate buffer (pH 4.5) and 35% of acetonitrile as mobile phase and the mobile phase comprised of ammonium formate (5.0 mM) and 85% of acetonitrile as mobile phase. In another approach, the separation was achieved using Hypersil GOLD C_{18} column, with acetonitrile and formic acid (0.1%) in aqueous medium by ultra-performance liquid chromatography (UPLC) partitioning using C_{18} column. The eluent was further studied with multiple regression monitoring tandem quadrupole mass spectrometer [11]. Hypersil GOLD C_{18} column (50 mm 3.0 mm, 5 µm) column using 15% of ammonium formate (5.0 mM) and 85% of acetonitrile as eluent in acidic conditions (pH 4.5) was employed for the resolution of hydrochlorothiazide and lisinopril [12].

Antihypertensive (β-blockers) and broncho agonist in plasma and urine were extracted using determined using solid phase microextraction method, later, they were analyzed using pentamorphosylanthonydary and the mobile phase comprised of acetonitrile and water [13]. Telmisartan and pioglitazone were estimated using C_{18} (50 mm 4.6 mm 5 µm) column with methanol-ammonium formate (1:1 ratio) mobile phase and organic phase consists of 20:80 (v/v) formic acid (0.1%) and acetonitrile used as eluent [14]. The separation was achieved using Hypersil GOLD C_{18} column, with acetonitrile and formic acid (0.1%) in aqueous medium by ultra-performance liquid chromatography (UPLC) partitioning using C_{18} column. The eluent was further studied with multiple regression monitoring tandem quadrupole mass spectrometer [11]. Hypersil GOLD C_{18} column (50 mm 3.0 mm, 5 µm) column using 15% of ammonium formate (5.0 mM) and 85% of acetonitrile as eluent in acidic conditions (pH 4.5) was employed for the resolution of hydrochlorothiazide and lisinopril [12].
Chromatographic separation and validation of the combined tablet containing atorvastatin, metformin, and glimepiride reported using BEH C<sub>18</sub> column, and eluent consists of 600 mL of acetonitrile (60%) and phosphate buffer (pH 3) 400 mL (40%) [21]. UPLC with PDA detector was used for the development of method and validation for metformin and telmisartan in pure solid dosage form using column C<sub>18</sub> (150 × 4.6 ID) 5 μm, with sodium dihydrogen phosphate buffer (NaH<sub>2</sub>PO<sub>4</sub>) and acetonitrile (60:40v/v) as mobile phase [22]. In another study, metformin hydrochloride, ramipril, and glimepiride were separated using mobile phase of 0.02 M KH<sub>2</sub>PO<sub>4</sub> buffer and methanol (150:850) using Hypersil BDS C<sub>18</sub> column [23].

The estimation of nifedipine, nateglinide, and lowastatin was achieved simultaneously by Millennium C<sub>18</sub> column and 40% of 10 mM phosphate buffer (pH 3.5) and 60% acetonitrile as eluent [24]. Estimation of antihypertensive and antidiabetic drugs using RP-HPLC separation was achieved by organic and aqueous phase (60:40, v/v) [25]. Degradation studies of valsartan were performed using isocratic HPLC method with C<sub>18</sub> reverse stationary phase, with 70% methanol and 30% water (pH 7.2) as mobile phase was used in acid hydrolysis stability-indicating assay [26]. Analysis of glibenclamide, metformin, captopril, and pioglitazone in API was reported with 70% methanol and 30% water using Hypersil ODS C<sub>18</sub> column [27]. Glibenclamide, amlodipine, atorvastatin, and metformin in human plasma were separated and validated by HPLC-UV method [28]. Antidiabetic and antihypertensive drugs were studied for stability indicating assay using RP HPLC method; the separation was achieved isocratically on a C<sub>18</sub> column [29]. In a similar study, the combination of metformin and telmisartan was analyzed and validated by RP-HPLC method in bulk and in formulations [30].

The estimation of metformin hydrochloride, glimepiride, and atorvastatin calcium was carried out simultaneously in bulk and combined dosage form by stability indicating RP-HPLC technique [31]. Telmisartan and metformin hydrochloride were estimated by RP-HPLC method in bulk and synthetic mixture [32]. The interaction of nifedipine and metformin in hypertension with type II diabetic patients was studied using ODS Hyperil C<sub>18</sub> column, using acetonitrile, 25 mM KH<sub>2</sub>PO<sub>4</sub>, and methanol as eluents [33].

Pioglitazone and telmisartan in a formulation were estimated by RP-HPLC technique using 35% of 0.5% triethylamine and 65% of acetonitrile [34]. High-performance thin-layer chromatography (HPTLC) technique was used for the estimation of metformin, glimepiride, and atorvastatin in fixed dosage combination using water: methanol:ammonium sulfate as mobile phase [35]. In a similar study, the determination of antidiabetic and antihypertensive drugs in pharmaceutical formulations was analyzed by RP-LC with Lichrocart C<sub>18</sub> as a stationary phase and methanol:water:orthophosphoric acid (75:25:0.2) as mobile phase [36]. Simultaneous estimation of atorvastatin, metformin, and glimepiride in the formulation was carried out by RP-HPLC technique, and the separation was achieved by Hibar C<sub>18</sub> as stationary phase using 40% of AGN and 60% ammonium acetate (10 mM, pH 3.0, adjusted using acetic acid) as eluent, respectively [37].

CONCLUSION

Chromatographic methods are considered to be rapid tools for qualitative and quantitative analysis of newer chemical entities in pharmaceuticals. In recent times, there were several new drug molecules that were introduced in to the market, but always there exists a lag in establishing a defined protocol for the analysis of impurities, their identification and the development of assay methods. Hence, this review attempts to collate various analytical techniques that were developed and validated for the estimation of few important antidiabetic and antihypertensive drugs either in pure, individually or combined with other pharmaceutical dosage form by HPLC, LC-MS/MS, and HPTLC techniques.

ACKNOWLEDGMENTS

The authors are thankful to the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), for providing the necessary library facilities, infrastructure, and equipments for carrying out the research work.

AUTHORS' CONTRIBUTIONS

The authors SV and HG had contributed equally towards the collection of literature and preparation of the manuscript.

REFERENCES