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Literature Review: Analysis and Validation of the Effectiveness of Antidiabetic Drugs in the Biological Matrix Using the HPLC (High Performance Liquid Chromatography) Method

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HPLC, Antidiabetes, Method validation

ABSTRACT
High-Performance Liquid Chromatography (HPLC) is a widely used analytical method for the separation, identification, and quantification of compounds in complex biological matrices, such as plasma, serum, and urine. This article discusses the analysis and validation of the effectiveness of antidiabetic drugs using HPLC, focusing on sample preparation, column selection, mobile phase, and detectors used. Method validation is performed to ensure parameters such as specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) are met. Literature study from 18 journals showed that HPLC is a sensitive, specific, and efficient method for antidiabetic drug analysis. Preparation techniques such as protein precipitation and solid-phase extraction provide optimal results in minimizing biological matrix interferences. In addition, the Photodiode Array (PDA) detector proved superior in terms of sensitivity and analyzability at low concentrations compared to other detectors. By fulfilling all validation parameters, HPLC can be relied upon as a primary method for the analysis of antidiabetic drugs in biological matrices.

INTRODUCTION

Diabetes mellitus (DM) is one of the chronic metabolic diseases that continues to increase globally and has become one of the major health problems in many countries, including Indonesia. Based on data from the World Health Organization (WHO) in 2020, the prevalence of diabetes is expected to continue to increase along with changes in lifestyle, diet, and genetic factors that affect the development of this disease. DM is characterized by a disorder of carbohydrate metabolism that causes blood glucose levels to become high (hyperglycemia). Uncontrolled glucose levels in the long term can trigger a variety of serious complications, such as damage to the cardiovascular, kidney, nervous systems, as well as blindness

Diabetes management involves various types of therapy, one of which is the administration of anti-diabetic drugs to regulate blood glucose levels within normal limits. Different types of drugs have become available for diabetes therapy, including insulin, sulfonylurea, metformin, glitazone, as well as DPP-4 inhibitors, each of which has a different mechanism of action in lowering blood glucose levels (Antar et al., 2023) Although these drug therapies can be effective, their success is highly dependent on the selection of the right dose,

the suitability of the drug to the patient's condition, as well as monitoring of the drug's levels in blood plasma. Proper monitoring of the concentration of drugs in plasma becomes crucial, as too low concentrations of drugs can reduce the effectiveness of therapy, while too high concentrations can lead to side effects or even poisoning (Ramakrishna et al., 2021)

To ensure the effectiveness of therapy, the analysis of drug concentrations in the biological matrix is an important step. The effectiveness of antidiabetic therapy should be thoroughly tested through various analysis methods to ensure that the drugs used are safe and have optimal pharmacological potential. One of the most widely used analytical methods in determining drug concentrations in the biological matrix is High Performance Liquid Chromatography (HPLC). HPLC is a highly sensitive chromatography technique and can result in clear separation of various chemical components in a sample, including drugs. In the context of anti-diabetic drug testing, HPLC allows for the determination of drug concentrations with a high degree of precision and can be used to analyze multiple drugs at once in a single plasma sample. Therefore, HPLC is a very useful method in the development and evaluation of drug therapies, both in research laboratories and in clinical supervision (Sankar et al., 2021)

Although HPLC is a very effective and frequently used method, to ensure that the results of such analyses are reliable and applicable in clinical practice, the validation of the HPLC method used must be thorough. HPLC method validation aims not only to ensure the accuracy and precision of measurement results, but also to test the limit of detection (LOD), limit of quantification (LOQ), and specificity, linearity, and resistance of methods to variations in analysis conditions. Therefore, the validation of the HPLC method in the context of the analysis of anti-diabetic drugs must include several very important parameters, all of which play a role in determining whether the method can be used to generate accountable data in the management of diabetes therapy (Gedawy et al., 2018)

This study aims to study the analysis of the use of HPLC in determining the levels of antidiabetic drugs contained in the biological matrix to optimize easy, sensitive, and time-efficient methods in the analysis of antidiabetic drugs in the biological matrix so that it can determine the monitoring of drug levels in patients and ensure the effectiveness of the drugs used.

RESEARCH METHOD

The data and information used in this literature come from journals, both national and international. Data and information search was carried out using the literature research method. Searches for journals accredited as primary libraries were searched for the keywords "application of HPLC in pharmaceuticals" and "analysis of anti-diabetic drugs with HPLC method" and "analysis of drug effectiveness in biological matrix with HPLC/KCKT". Search strategies were identified from relevant electronic databases, namely Google Scholar and PubMed with a range of years 2014-2024. The search results obtained were filtered based on relevant bibliographies so that 18 journals were obtained to be reviewed.

RESULT AND DISCUSSION

Table 1. Review Results of Antidiabetic Drug Analysis Methods on the Biological Matrix

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
Glibenklamide	Human blood plasma	SPE C18 and SPE-MIP acrylamide	Column: C18 150 x 4.6 mm, particle size 5 µm Flow rate: 1	SPE C18: 0.44022 µg/mL SPE MIP	SPE C18: 93-103% Acrylamide of Spay Mip Monomer: 97-	Hasanah et al., 2016

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
		monomers	mL/min Gerak phase: 55:45 v/v asetonitril dan trifluorode acid 0.1% Detector: UV (227 nm)	monomer acrylamide: 1.965276 µg/mL	108%	
Metformin	Human blood plasma	Liquid-liquid extraction	VP ODS C-18 column, column length 5 µm, 4.6 x 150 mm. Flow rate: 1 mL/min Motion phase: methanol:water (SDS 3 mM) Detector: UV (226 nm)	0,8 ppm	80-120%	Fernando et al., 2024
Metformin, Pioglitazone, dan Glimepiride	Human blood plasma	Protein precipitation	Column: C18 150 x 4.6 mm, particle size 5 µm Flow rate: 1 mL/min Phase of motion: MeOH : KH2PO4 (85:15) Detektor: Diode Array Detector (DAD) (235 nm)	Metformin: 0.18 µg/mL Pioglitazone: 0.894 µg/mL Glimepiride: 0.35 µg/mL	Metformin: 74-85% Pioglitazone: 80-85% Glimepiride: 79-86%	Sebaiy et al., 2020
Metformin dan Evogliptin	Human blood plasma	Protein precipitation	Wheel: Zodiac-100 C18 (150 × 4.6 mm, 5 µm) Flow rate: 1 mL/min Gerak phase: asetonitril:aquadest (70:30) Detektor: UV (230 nm for Metformin and 205 nm for Evogliptin)	Metformin: 1.34 µg/mL Evogliptin: 1.12 µg/mL	Metformin: 0,52% Eevogliptin: 0.84%	Kharabe et al., 2024
Glibenklamide	Human plasma and urine	Protein precipitation	Kolom: C18 (4.6 x 250 mm; 5 µm) Flow rate: 1 mL/min Gerak phase: aquadest:asetonitril (30:70) Detektor: Photodiode Array (PDA) (229 nm)	1.0 µg L−1	89,4-102,9%	Sabbaghi et al., 2024
Glipizides	Human urine	Magnetic solid-phase extraction	Column: Inertsil ODS-3 conventional (250 mm x 4.6 x 5.0 µm) (C18) Flow rate: 1.0	14.71 µg mL− 1	104.5%, 106.1%, and 97.1%	Gharsallah et al., 2024

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
			mL/min Phase of motion: Methanol: Trifloroaacetic acid (1.0%); ACN (17:53:30). Detektor: Diode Array Detector (DAD) (286 nm)			
Metformin Hydrochloride	Human blood plasma	Complex liquid-liquid extraction	Column: RP-18 (250 mm × 4.6 mm × 5 µm) Flow rate: 1.2 mL/min Gerak phase: phosfat buffer (70%) : acetonitril (30%) Detector: UV (233 nm)	0,18 µg/mL	96,159% - 104,905%	Pradana et al., 2023
Metformin Hydrochloride	Human blood plasma	Complex liquid-liquid extraction	Column: C-18 (250 mm × 4.6 mm, 5 µm) Flow rate: 1.3 mL/min Gerak phase: acetonitrile (34%) : aqueous (66%) Detector: UV-Vis (233 nm)	0,125 µg/mL	98% - 105,89%	Chhetri et al., 2014
Glimepiride, metformin, sitagliptin, Rosiglitazone dan Pioglitazone	Human blood urine and plasma	Sonicics	Column: C-18 (250 mm × 4.6 mm, 5µm) Flow rate: 1 mL/min Gerak phase: acetonitrile (40%) : phosphate buffer (40%) : methanol (20%) Detector: RP-HPLC UV (240 nm)	Metformin: 1.62 ng/mL Glimepiride:3.96 ng/mL Pioglitazone: 2.56 ng/mL Rosiglitazone: 2.33 ng/mL Sitagliptin: 4.55 ng/mL	Metformin: 100,12% Glimepiride: 98,22% Pioglitazone: 99.12% Rosiglitazone: 99,07% Sitagliptin: 98,42%	Sher et al., 2019
Linagliptin dan Metformin	Human blood plasma	Protein precipitation	Kolom: Reverse Phase Grace vyadyec genesis CN (150 mm × 4.6 mm, 4 µm). Flow rate: 1.0 mL/min Fase gerak: acetonitril : buffer dipotassium hydrogen phosphate (0.01M,	-	Linagliptin: 77, 39%-88.13% Metformin: 82,72%-92,13%	Pandya et al., 2014

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
			pH=7) (75:25). Detector: UV (237 nm)			
Omarigliptin, Metformin, Ezetimibe	Human blood plasma	Protein precipitation	Column: Hypersil BDS C18 (250 mm × 4.6 mm id, particle size 5 µm) Flow Rate: 0.814 mL/min Motion Phase: Methanol and potassium phosphate buffer dihydrogen (6.6 mM, pH 7) (67:33% v/v) Detektor: Diode Array Detector (DAD) (235 nm)	Omarigliptin: 0.2 µg/mL Metformin: 0.5 µg/mL Ezetimibe: 0.1 µg/mL	Omarigliptin: 96.8%-102.82% Metformin: 97.2%-101.21% Ezetimibe: 97.67%-104.15%	Magdy et al., 2023
Metformin, glibenclamide	Human blood plasma	Protein precipitation	Kolom: Waters Nova Pack Phenyl (150 mm x 4.6 mm, 5 µm) Flow rate: 1.0-1.5 mL/min Injection volume: 20 µL Phase of motion: 0.1% phosphoric acid (pH 3.0) and acetonitrile Detector: UV-Vis (227 nm)	Metformin: 10 µg/mL Glibenklamide: 50 µg/mL	Metformin: 92.6% Glibenclamide: 86.4%	Porwal & Talele, 2017
Glibenklamide	Human blood plasma	Get ready to MIP MAA	Column: Shimadzu (150 mm × 4.6 mm) Flow rate: 1 mL/min Gerak phase: asetonitril : TFA 0.1% (55:45 v/v) Detector: Uv-Vis (Dionex Ultimate 3000)	Glibenclamide : 1,733239 µg/mL.	92,28%; 106,02%; and 97.39%.	Rohayati et al., 2015
Metformin Hydrochloride & Vildagliptin	Human blood plasma	Protein precipitation	Wheel: Thermo Hypersil ODS C18 (5 µm, 4.6 mm × 250 mm). Motion phase: Mixture of methanol, acetonitrile, and phosphate buffer 5:30:65 (v/v), pH 3.5. Injection volume:	Metformin Hydrochloride : 6.57 µg/mL (plasma) Vildagliptin: 0.41 µg/mL (plasma)	Metformin Hydrochloride : 93.68% Vildagliptin:91.80%	Shakoor et al., 2020

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
			20 µL Flow rate: 0.8 mL/min Detector: UV/Vis at 212 nm wavelength			
Metformin & Saxagliptin	Human blood plasma	Protein precipitation	HPLC column: Enable C18 G (250 × 4.6 mm; particle size 5 µm). Motion Phase: Mixture of 0.05 M KH_2PO_4 buffer (pH 4.5):Methanol :Acetonitrile (60:20:20 (%v/v)). Flow Rate: 0.6 mL/min. Injection Volume: 10 µL. Detektor: UV detector (220 nm)	Metformin: 0.373 µg/mL Saxagliptin: 0.096 µg/mL	Metformin : 50% level: 99,71% 100% level: 99,79% 150% level: 99,03% Installment: 99.71 ± 0.47% Saxagliptin: 50% level: 99,94% 100% level: 100,54% 150% level: 99,95% Installment: 100.14 ± 0.90%	Prasad et al., 2015
Rosiglitazone	Human blood plasma	Protein preci pitation	Column KCKT: (8 x 100 mm 4-µm) Nova-Pak C18 Flow rate: 2.0 mL/min Fase gerak: 0.01 M dibasic potassium hydrogen phosphate (pH 6.5, adjusted with phosphoric acid) and acetonitrile (65:35, v:v) Detektor: Fluorescence (247 nm (excitation) and 367 nm (emission)	5 ng/mL	91%; 92%: 96%; 99%	Yusuf et al., 2015
Nateglinide	Human blood plasma	Vortex-assisted DLLME	Column KCKT: Hypersil BDSC-18 column (250 mm) Flow rate: 1 mL/min Fase gerak: phosphate buffer (10 mM, pH 2.5) and acetonitrile (35:65, v/v) Detector: UV-Vis (210 nm)		102.5 and 105.9%	Hammad et al., 2021
Empagliflozin and Linagliptin	Human blood plasma		HPLC column: Agilent C18 column (50 µm, 4.6×250 mm) Flow rate: 0.7 ml/min	Empagliflozin: 0.45 µg/mL Linagliptin: 0.31 µg/mL	Empagliflozin: 99.70% Linagliptin: 99.80%	Patil & Gupta, 2024

Obat Antidiabetes	Matrix	Sample Preparation	Parameter HPLC	LOQ	Recovery	Ref.
			Fase gerak: methanol and 0.1% ortho phosphoric acid in water (45:55, pH 2.5) Detektor: Diode-Array-Detector (248 nm)			

Table 2. Results of the Parameter System for Validating Antidiabetic Drug Analysis Methods in the Biological Matrix

Sample	Specificity	Linearitas (R2)	Accuracy (*%recovery;* %)	Precision (%)	LOD (µg/mL)	LOQ (µg/mL)	Ref.
Glibenklamide	√	SPE C-18: 0,9984 SPE MIP monomer akrilamid: 0,9998	EPS C-18: 93.16814 - 103.0133 SPE MIPA acrylic monomer: 97.73067124 - 109.7588834	EPS C-18: 4.909356 - 16.90148 SPE MIPA acrylic monomer: 1.07651223 - 7.730881028	EPS C-18: 0.589583 SPE MIPA acrylamide monomer: 0.132066	EPS C-18: 1.965276 SPE MIPA acrylic monomer: 0.44022	Hasanah *et al.,* 2016
Metformin	√	0,9979	237,3 - 2245,6	Intra-day: 6,72 - 19,7	0,24	0,8	Fernando *et al.,* 2024
Metformin (MF) dan Evogliptin (EVG)	√	MTF: 0,9999 EVG: 1	MTF: 100 EVG: 100	MTF: 1.4 EVG: 1.4	MTF: 0,34 EVG: 0,40	MTF: 1,12 EVG: 1,34	Kharabe *et al.,* 2024
Glibenklamide	-	0,9965	89,4-102,9	-	0,3	1,0	Sabbaghi *et al.,* 2024
Glipizides	-	0,9942	97,1 - 106,1	4,2 - 6,7	$14{,}71\times10^{-3}$	4.38×10^{-3}	Gharsallah *et al.,* 2024
Metformin Hydrochloride	√	0,9998	100,96	Intra-day: 2,008 – 6,542 Inter-day: 1,782 – 5,305	-	0.18	Pradana *et al.,* 2023
Metformin Hydrochloride	√	0,9951	98 - 105,89	6,97	0,062	0,125	Chhetri *et al.,* 2014
Glimepiride (GP),	√	GP: 0,9992 MF: 0,9998	96-101,8	1,5-2,4	GP: $0.88\times^{10-3}$	GP: 3.96×10-3	Sher *et al.,* 2019

Sample	Specificity	Linearitas (R2)	Accuracy (*%recovery;* %)	Precision (%)	LOD (µg/mL)	LOQ (µg/mL)	Ref.
metformin (MF), Sitagliptin (SG), Rosiglitazone (RG) dan Pioglitazone (PG)		SG: 0,9993 RG: 0,9997 PG: 0,9995			MF: 0.52×10-3 DC 0.90×10-3 RG: $0,73\times10^{-3}$ PG: $0,66\times10^{-3}$	MF: 1.6×10-3 DC 4.5×10-3 RG: $2,33\times10^{-3}$ PG: $2,56\times10^{-3}$	
Linagliptin (LGP) dan Metformin (MF)	√	LGP: 0,9997 MF: 0,9999	LGP: 77.39-88.13 MF: 82.72-92.13	Intra-day LGP: 1,325-9.823 Inter-day:1,632-7,708 MF Intra-day: 0,248-3,382 Inter-day: 0,784-2,883	-	LGP & MF: 1	Pandya et al., 2014
Omarigliptin (OGP), Metformin (MF), Ezetimibe (ETB)	√	OGP, MF, ETB: 0,9999	OGP: 96,8-102,82 MF: 97.2-101.21 ETB: 97.67-104.15	OGP: 1,972 MF: 1,365 ETB: 1,825	OGP: 0,02 MF: 0,18 ETB: 0.02	OGP: 0,06 MF: 0,50 ETB: 0.06	Magdy et al., 2023
Metformin (MF), Glibenclamide (GLB)	√	-	MF: 93.54 - 86.98 CAP:	Intraday: 2,99 - 15,61 Inter-day: 3,65 - 18,57	-	-	Porwal & Talele., 2017
Glibenklamide	√	EPS C-18: 0.996 SPE-MIP COUNTRY: 0.998	SPE C-18: 95.99%; 99,2%; and 105.17% SPE-MIP MAA: 92.28%; 106,02%; and 97.39%.	SPE C-18: 16.90; 10.92 and 4,91; SPE-MIP MAA: 1.95; 4.54 and 4.56.	EPS C-18: 0.589583 SPE-MIP MAA: 0.519972	EPS C-18: 1.965277 SPE-MIP COUNTRY: 1.733239	Rohayati *et al.,* 2015
Metformin Hydrochloride (MFH) & Vildagliptin (VDP)	√	MFH: 0.9913 VDP: 0.9902	MFH: 99,83–100,22 DV: 100.71–102.30	MFH: 0.22 - 1.27 VDP: 1.12 - 1.43	MFH: 2.18 and 6.55; VDP: 0.13 and 0.38	MFH: 6,57 VDP: 0,41	Shakoor *et al.,* 2020

Sample	Specificity	Linearitas (R2)	Accuracy (*%recovery;* %)	Precision (%)	LOD (µg/mL)	LOQ (µg/mL)	Ref.
Metformin Hydrochloride	√	0.9951	98 - 105,89	Intra-day: 0,21 – 4,54 Inter-day: 2,30 – 6,97	0,062	0,125 µg/mL	Chhetri *et al.,* 2014
Metformin (MF), Pioglitazone (PG), dan Glimepiride (GP)	√	MF, PG, GP: 0,999	MF: 98.25-101.01 PG: 98,84-101,51 GP: 98.60-100.82	MF Intra-day: 0,08 – 0,14 Inter-day: 0,04 – 0,07 PG Intra-day: 0,29 – 1,16 Inter-day: 0,11 – 0,93 GP Intra-day: 0,10 – 0,36 Inter-day: 0,10 – 0,30	MF: 0,05 PG: 0,26 GP: 0,10	MF: 0,18 PG: 0,89 GP: 0,35	Sebaiy *et al.,* 2018
Metformin (MF) & Saxagliptin (SGP)	√	MF: 0,998 SGP: 0,999	MF: 98.31-100.65 SGP: 98.87-101.62	MF Intra-day: 0,167 – 1,453 Inter-day: 0,144 – 1,396 SGP Intra-day: 0,452 – 0,871 Inter-day: 0,468 – 1,329	MF: 0,112 SGP: 0,029	MF: 0,373 SGP: 0,096	Prasad *et al.,* 2015
Rosiglitazone	-	0,9993	91 - 99	Intra-day: 1,9 - 3,1 Inter-day: 3,1 - 7,7	$2{,}5\times10^{-3}$	5×10^{-3}	Yusuf *et al*., 2015
Nateglinide	√	0,9992	102,5 - 105,9	Intra-day: 3,45 – 5,64 Inter-day: 0,86 – 3,25	15×10^{-3}	-	Hammad *et al*. 2021
Empagliflozin (EGZ) and Linagliptin (LGP)	√	0,999	EGZ: 99.70 LGP: 99,80	EGZ: 0.27 LGP: 0,40	EGF: 0.15 LGP: 0,10	EG: 0.45 LGP: 0,31	Patil & Gupta, 2024

High-Performance Liquid Chromatography (HPLC) is a common instrument used in the identification and determination of various natural and synthetic compounds in a specific, efficient, and fast manner. HPLC has been widely used in fields such as pharmaceutical, environmental, forensics, food and flavor, clinical, and many other fields in terms of qualitative and quantitative analysis (Chawla & Chaudhary, 2019). These advantages make HPLC also widely used for the need for drug analysis in a biological matrix. HPLC is used primarily for the separation, identification, and quantification of a compound in a complex biological matrix. HPLC is the most accurate method that is widely used in the quantitative and qualitative analysis of a drug active substance and is used in determining the stability of a drug (Rao et al., 2015).

The use of HPLC in the analysis of drugs and metabolites in biological matrices, particularly plasma, serum, and urine is common. Blood plasma or serum contains many endogenous compounds that often have much greater concentrations compared to analytes. A low concentration of analytes in a drug case will make it difficult to measure because sometimes structurally endogenous compounds have similarities to the compounds to be measured. In addition, the binding of the drug to plasma proteins can also occur, this can reduce the number of compounds to be measured.

In analyzing drugs and metabolites in a body fluid using HPLC, there are things that must be considered. First, the body fluid sample to be analyzed is extracted first due to the complex nature of body fluids. The resulting extraction must also be relatively clean and the separation system must be able to separate the desired drug from the extraction result. Then other things that must be considered include the selection of a good detector, a good stationary phase, a suitable motion phase, and adequate programming during the separation process (Nikolin et al., 2004).

HPLC utilizes two operational phases, namely the motion phase and the silent phase. The motion phase consists of liquids or solvents that play a role in transporting mixed components to the detector, while the silent phase is a phase that remains in the column, consisting of particles with small pores and having a large surface area. The silent phase used in the analysis of antidiabetic drugs with HPLC in the journals we reviewed mostly used C18 or octadecil silica (ODS). The C18 column is one of the most widely used silent phases in liquid chromatography engineering due to its superior ability to separate compounds with varying degrees of polarity, ranging from low to high. This advantage is due to the silky structure that is bound to long alkyl chains so that it enables strong hydrophobic interactions with the target molecules (Aulia et al., 2016).

In addition to C18, a type of phenyl column is also used. The phenyl-silica column has a phenyl group that is covalently bonded to the silica particles. This phenyl group provides special characteristics that allow π-π interactions between the dormant phase and the target compound, making it very effective for separating aromatic compounds as well as compounds that have double bonds, such as natural fatty acids or polycyclic aromatic compounds (Harmita et al., 2019).

HPLC columns vary in length, internal diameter, as well as still-phase particle sizes to meet diverse analysis needs. In general, HPLC columns are in the shape of a straight tube made from materials that are resistant to high pressure and chemicals, such as stainless steel, glass, or polymers. The length of the columns generally varies up to 25 cm (250 mm) (Fiorelia et al., 2022). The column lengths used in the above journals vary from 100 mm, 150 mm, and 250 mm, so these columns are well suited for the separation of complex compounds with optimal efficiency.

The internal diameter (ID) of the HPLC column is an important aspect that determines the quantity of analytes that can be loaded into the column and also affects the sensitivity of the analysis. Columns with smaller internal diameters have an advantage in terms of analysis sensitivity. Smaller column sizes allow for faster and more efficient phase flow of motion, which often leads to a reduction in the amount of solvent required. The internal diameter mentioned above has a size of 4.6 mm which is in accordance with what Fiorelia et al. (2022) mentioned, that the internal diameter ranges up to 5 mm.

The dimensions and particle size of columns affect the performance of columns. Columns with smaller dimensions or finer particle sizes will improve separation efficiency with shorter analysis times. The smaller the size of the column filling particles, the efficiency and resolution of the separation will increase, but higher pressure is needed to maintain it (Susanti et al., 2018). Referring to particle size data from journals obtained, many use particle sizes of 5 μm and also 4 μm. According to Fiorelia et al. (2022), HPLC columns with packed columns have a particle size between 3-10 μm.

HPLC (High-Performance Liquid Chromatography) is a widely used method in the quantitative analysis of medicinal products. The working principle of HPLC involves injecting a sample into a column containing a stationary phase, where the motion phase is pumped using high pressure towards the column. Sample separation occurs through the interaction between the motion phase and the stationary phase. The motion phase is responsible for sorting out the sample so that it can reach the detector. Reversed Phase HPLC (RP-HPLC) uses a nonpolar phase of motion. The pKa and pH regulation of the analyzer serves to determine and ensure the retention time and shape of the chromatogram peak. The addition of solvents and the phase of motion can affect the shape of the peak. Based on the results of the review, most previous studies used a mixture of acetonitrile and dapar phosphate with varying ratios as the phase of motion. ?

In the analysis of metformin in blood plasma using RP-HPLC and HPLC, the general motion phases used consist of acetonitrile as a nonpolar solvent and dapar phosphate as a polar solvent. Acetonitrile is an aprotic polar compound, while methanol, which is also used in other studies, is protic polar. Acetonitrile has several advantages over methanol, such as higher elution ability, higher boiling point, and lower UV cut-off. Acetonitrile has a low viscosity that helps reduce the backstress on the system as well as results in a better chromatogram peak shape. In addition, this solvent supports UV detection at low wavelengths and easily forms binary mixtures with water. On the other hand, methanol is more economical and harmless than acetonitrile. Methanol is also more polar, thus reducing the likelihood of precipitation of drug compounds in the motion phase buffer solution. Polar-polar or ionic interactions between methanol and analytes often provide better selectivity, especially for polar compounds, although they require a longer analysis time (Sharma et al., 2022).

Sample preparation is one of the important steps that affect the success of antidiabetic drug analysis using High-Performance Liquid Chromatography (HPLC). Biological matrices such as blood, urine, and plasma often contain components that can interfere with or interact with the compounds being analyzed, such as proteins, fats, or other metabolites. Therefore, proper sample preparation techniques should be used to minimize this interference and ensure accurate and reliable analysis results (Muralidhalan et al., 2018).

The various sample preparation techniques used in the analysis of antidiabetic drugs with HPLC in the journals we reviewed showed their respective advantages in terms of efficiency, selectivity, and accuracy. Based on the results of the analysis from the journal, sample preparation by protein precipitation is the simplest and fastest method, but it is less selective. Liquid-liquid Extraction, Solid-Phase Extraction (SPE), and advanced techniques

such as Molecularly Imprinted Polymer (MIP) and Magnetic Solid Phase Extraction (MSPE) offer higher selectivity and sensitivity. The selection of the right sample preparation technique depends on the properties of the drug compound being analyzed and the type of biological matrix used. In this study, the combination of several extraction methods, such as SPE and MIP, provided the best results in terms of accuracy and sensitivity, making it a top choice in the analysis of antidiabetic drugs.

The development of HPLC is in line with the detectors used. According to Suprianto (2023), detectors in HPLC function to detect sample components in the flow that comes out of the column. A good detector must have the following characteristics, namely have high sensitivity and fast response to all solutes, be stable in operation, not affected by changes in temperature and flow speed of the moving phase, have a small volume cell so that it is able to minimize band expansion, and provide a linear response to solute and inert concentrations of solutes. In conducting an HPLC analysis, it is important to consider not only speed, sensitivity, and resolution but also the cost of selecting a good detector. The use of different detectors has their own reasons for use and advantages. The most commonly used detectors are UV, UV-Vis, PDA, DAD, and Fluorescence for the analysis and validation of the effectiveness of antidiabetic drugs in biological matrices using HPLC. According to Sholihah et al., (2021) UV detectors are detectors that are commonly used in HPLC analysis. However, these detectors are less sensitive when used to detect small amounts of analytes. In the literature review, a total of 10 journals or articles used UV detectors. Fluorescence detectors have the advantage of higher sensitivity and selectivity compared to UV detectors. In the review of this article, a total of 1 journal used fluorescence detectors, namely in studies (Yusuf et al., 2015) for the detection of antidiabetic drugs in the biological matrix.

Diode Array (DAD) detectors, also known as Photodiode Arrays (PDAs), are widely used chromatography tools, especially in high-performance liquid chromatography (KCKT). Its main function is to detect and measure simultaneously a variety of sample compounds with varying light-absorption wavelengths, in the ultraviolet and visible (UV-VIS) ranges. Photodiode Array (PDA) detectors are UV detectors with several features such as having a detection speed 300-400 times better than Photomultiplier Tube (PMT) detectors, a wavelength measurement range of 200-650 nm, no mechanical movement, and only one focusing lens. The PDA detector is also capable of displaying a three-dimensional chromatogram which will be very helpful in determining the purity of the chromatogram peak (Oxvyena, 2022).

In some of the detectors mentioned above, the one that has more ability to analyze antidiabetic drugs in the biological matrix is the PDA detector. According to Bethary's (2022) research, the use of UV detectors is not as good as Photodiode Array Detectors (PDAs) in terms of specificity. These detectors can provide a set of chromatograms simultaneously at different wavelengths in a single run. This is also shown in Table 1. where PDA detectors showed better ability in terms of Limit of Quantification (LOQ) and recovery compared to UV and fluorescence detectors for some of the antidiabetic drugs analyzed. PDAs have a very low LOQ for some antidiabetic drugs, which indicates high sensitivity in detecting these drugs at very low conditions. In the table, although the recovery for PDA has varying values, some analyses have fairly good recovery, although not as optimal as UV detectors. UV detectors have a fairly good recovery result, but in terms of LOQ value, UV detectors have a higher LOQ value than PDAs. Overall, PDA detectors can be considered the best choice for antidiabetic drug analysis on biological matrices due to their ability to detect at low concentrations and provide additional spectral information that can aid in more accurate identification of compounds.

Flow rate is an important parameter in the HPLC method because it affects various aspects of analysis performance, such as separation time, efficiency, resolution, and sensitivity (Rosydiati & Saleh, 2019). Based on the results of a review of 18 journals, the flow rate used for the analysis of antidiabetic drugs in the biological matrix varies from 0.6 mL/min to 2.0 mL/min, depending on the type of column, the properties of the analyte, and the composition of the phase of motion. Most studies used a flow rate of 1.0 mL/min, which is considered the optimal standard. This rate is widely used for C18-based columns with a length of 150–250 mm and a particle size of 5 μm, as it provides a balance between efficient analysis time and adequate separation.

Some studies used lower flow rates, such as 0.6 mL/min, as was done by Prasad et al. (2015) for the combination analysis of metformin and saxagliptin. This lower flow rate is used to increase the contact time between the analyte and the stationary phase, resulting in better separation resolution. In addition, the use of low flow rates also reduces the stress on HPLC systems, which is beneficial when analyzing complex compounds or complex matrices.

On the other hand, Yusuf et al. (2015) used a high flow rate of up to 2.0 mL/min for the analysis of rosiglitazone in human blood plasma. High flow rates speed up analysis time, but can reduce resolution if not balanced with motion phase and column optimization. Therefore, high flow rates are more often applied to analyses with one or fewer analytes, where complex separation is not a priority. The composition of the motion phase also has a big influence on the choice of flow rate. Most studies use solvent mixtures such as acetonitrile and phosphate buffers, where the viscosity of the mixture affects the column pressure. A flow rate of 1.0 mL/min is generally chosen because it provides system stability without causing overpressure, especially when using low-viscosity moving phases such as acetonitrile-water mixtures.

The validation of an analysis method is evidenced by the results of specificity tests, calibration curves, detection limit (LOD) and quantification limit (LOQ) tests, as well as precision and precision tests (Fernando et al., 2024). The assessment of certain parameters based on the results of laboratory experiments is known as analysis method validation. This is done to ensure that the analysis method has achieved its purpose and meets the requirements for use. Validation of the analysis method is necessary because it is to ensure that the performance parameters can solve the analysis problem (Arikalang et al., 2018).

The various research results that have been presented by various researchers in Table 1. confirmed the successful development and validation of the high-performance liquid chromatography (KCKT) method in analyzing antidiabetic drugs in human blood plasma. The HPLC method used in this study has undergone validation tests according to international guidelines, such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). According to Table 2. Describing the results of the validation of the analysis method, it is seen that various validation parameters, such as linearity, accuracy, precision, specificity, detection limits, and quantification, have been assessed and meet the requirements standards.

Specificity is the ability to properly assess the analyte and provide precise information about the content of the analyte or the possible analyte in a sample even if the sample consists of other components such as impurities, degradation products, and matrices. The specificity of the analyte in the KCKT method is indicated by the analyte's chromatogram peak that is not disturbed by the peak of the other components. This method should also be able to produce a good separation between the analyte and the other components of the matrix. To determine the selectivity of the method used, it can be seen from the power of separation (resolution) of the two peaks. When the resolution of the analysis method is greater than 1.5, the method is considered specific (Yuwono et al., 2021; Rohayati et al., 2015).

The results showed that previous researchers had developed a KCKT analysis method to determine the level of antidiabetic drugs in human blood plasma and had met the requirements for the acceptance of the specificity parameter, i.e. the result with a resolution greater than 1.5.

The next parameter of analysis is linearity. Linearity is known for its ability (within a certain range) to obtain test results that are directly proportional to the concentration or number of analytes in a sample. Linearity testing is performed by creating calibration curves with various sample concentrations, which are based on sample concentration and absorbance data. Furthermore, the regression equation and correlation coefficient (r) are calculated from the data. The result of the correlation coefficient (r) must be at least 0.99 according to the existing literature (Wulansari et al., 2020; Rochman, A., 2016).

The results of the study show that the KCKT analysis method made by the previous researcher to determine the level of antidiabetic drugs in the biological matrix has met the requirements for the acceptance of the linearity parameter. The overall data obtained shows linearity, which should be more than 0.99. This shows that this developed analysis method will provide an overall linear analysis result.

Precision is a measure of how close the results of an analysis are to a particular condition and treatment. Precision testing can be based on three factors: repeatability, intermediate accuracy, and reproducibility. For medium precision, precision testing can be performed intra-day and inter-day. Intra-day testing is conducted on a single day (morning, afternoon, and evening) with the same conditions, equipment, and treatments, while inter-day testing is performed on three consecutive days with different conditions, equipment, and treatments. Precision acceptability is based on a relative standard deviation (RSD), with an RSD value of no more than 2% (Annuryanti et al., 2018; Armin et al., 2015). The precision value obtained must be in accordance with the requirements, which is expressed by the KV value <15% for the middle concentration and <20% for LLOQ (Rohayati et al., 2015).

According to the results of the study, Table 2, all precision results show that the KCKT analysis method made by the previous researcher to determine the level of antidiabetic drugs in the biological matrix has met the requirements for the acceptance of precision parameters. The overall data obtained showed precision, where the data obtained was stated, with a KV (Coefficient of Variation) value of <15% for the middle concentration and <20% for LLOQ. This shows that the analysis method developed will provide an overall precise analysis result.

The accuracy parameter, also known as accuracy, is an analysis method validation parameter that indicates the proximity of the results between the analysis level (measured value) and the actual analytic level (the accepted value is correct). The percentage of recovery (percentage recovery) of the test analysis results compared to the actual analyte levels in the matrix component. The acceptable percentage requirement for repossession is 97-103%, according to AOAC (2016) (Harmono, 2020; Amin et al., 2016). However, for a biological sample analysis method, it is declared accurate if it has a % recovery value, namely 85−115% and 80−120% for LLOQ (Hasanah, 2016).

Based on the results of the literature study, it is shown that the development of the HPLC/KCKT analysis method for determining the level of antidiabetic drugs in the biological matrix by previous researchers has met the requirements for the acceptance of accuracy parameters. Except for the journals written by Fernando et al. (2024) and Pandya et al. (2014). The results of these two journals have not been able to meet the accuracy requirements because they exceed the specified requirements (results are in the range of 237.3 - 2245.6), for the journal Pandya et al. (2014) some of the data results have met the requirements and some have not met the requirements (77.39).

LOQ is the lowest analyte concentration that can be accurately and thoroughly quantified, while LOD is the smallest analyte concentration that can be read and provide a significant reaction compared to the reaction from the results of blank or noise analysis (Hasan et al., 2021). The strong relationship between the quantization limit and the detection limit makes them inseparable. There is no significant difference in the way the two are practically evaluated. Riyanto (2014) stated that the only thing that distinguishes the two is the type of quantitative data collected. LOD and LOQ are absolutely determined if the analyzed analyte has a relatively small concentration as in the biological matrix (Rohayati et al., 2015). In the results of research journals related to the parameters of LOD and LOQ that were presented, the results were varied. The results of LOD and LOQ greatly affect their relationship with obtaining precision and accuracy results.

CONCLUSION

Based on the analysis of 18 national and international articles, it can be concluded that High-Performance Liquid Chromatography (HPLC) is an effective and accurate method for the separation, identification, and quantification of compounds in complex biological matrices, such as plasma, serum, and urine, particularly in the analysis of antidiabetic drugs. This method is able to overcome challenges such as interference of endogenous compounds and drug binding to plasma proteins. Proper sample preparation, through extraction techniques such as protein precipitation, liquid-liquid extraction, and solid-phase extraction, is essential to minimize interference and improve accuracy. The selection of the appropriate silent phase, motion phase, and detectors, especially Photodiode Array (PDA) detectors, also contributes to the success of the analysis. HPLC method validation is necessary to ensure compliance with standards of specificity, linearity, precision, accuracy, and detection and quantification limits. The results of the study showed that the developed HPLC method met all the necessary validation parameters, so it was reliable for the analysis of antidiabetic drugs in the biological matrix.

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