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METHOD DEVELOPMENT AND VALIDATION OF ANTI-OBESITY DRUGS USING MODERN ANALYTICAL TOOLS: A COMPREHENSIVE REVIEW

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ABSTRACT
Obesity, characterized by excessive body fat accumulation and a Body Mass Index (BMI) of 30 or higher, has emerged as a critical global health concern. It contributes significantly to the risk of developing chronic diseases and has seen a steep rise in prevalence worldwide, including in India. This review article provides a comprehensive overview of obesity, its classifications, causes, and global trends. Emphasis is placed on the analytical method development of anti-obesity drugs, following ICH Q14 guidelines. Various modern analytical techniques—such as UV spectroscopy, HPLC, HPLC-MS/MS, gas chromatography, and HPTLC—are discussed in detail for their application in the quantification and assessment of drugs like Orlistat, Cetilistat, Semaglutide, etc. Each method is evaluated based on parameters like solvent, wavelength, retention time, precision, and recovery, highlighting their reliability and specificity in drug analysis. This work aims to support the standardization and optimization of pharmaceutical analysis to improve therapeutic outcomes in obesity treatment. Analytical method development, validation, and transfer are essential elements of any pharmaceutical development program. effective method development ensures that laboratory resources are optimised while methods meet the purpose required at each stage of drug development. With the increasing complexity of more than drug formulations, especially for Anti-Obesity drugs, there is a pressing need for reliable and reproducible analytical methods.

KEYWORDS: Obesity, Anti-obesity, Analytical method development, Method Validation, ICH, Analytical techniques, Regulatory requirements.

1. INTRODUCTION

Obesity
Obesity is commonly defined as having too much body fat. A BMI [Body mass index] of 30 or higher is the usual benchmark for obesity in adults. Obesity increases the risk of serious medical conditions. Treatments include changing what you eat, adding activity, and improving mental health.

1.1 Symptoms of obesity
While obesity is a disease, it doesn't cause any specific symptoms. A healthcare provider may define obesity by calculating the body mass index and body shape.

1.2 BMI classifications
They are classified into:
1. **Class I obesity:** BMI 30 to less than 35 kg/m² (kilograms per square meter).
2. **Class II obesity:** BMI 35 to less than 40 kg/m².
3. **Class III obesity:** BMI More than 4 kg/m².

1.3 Causes of obesity
Certain medications, Disability, Eating habits, Genetics, Lack of physical activity, Lack of sleep, Stress, and Underlying health issues.[1]

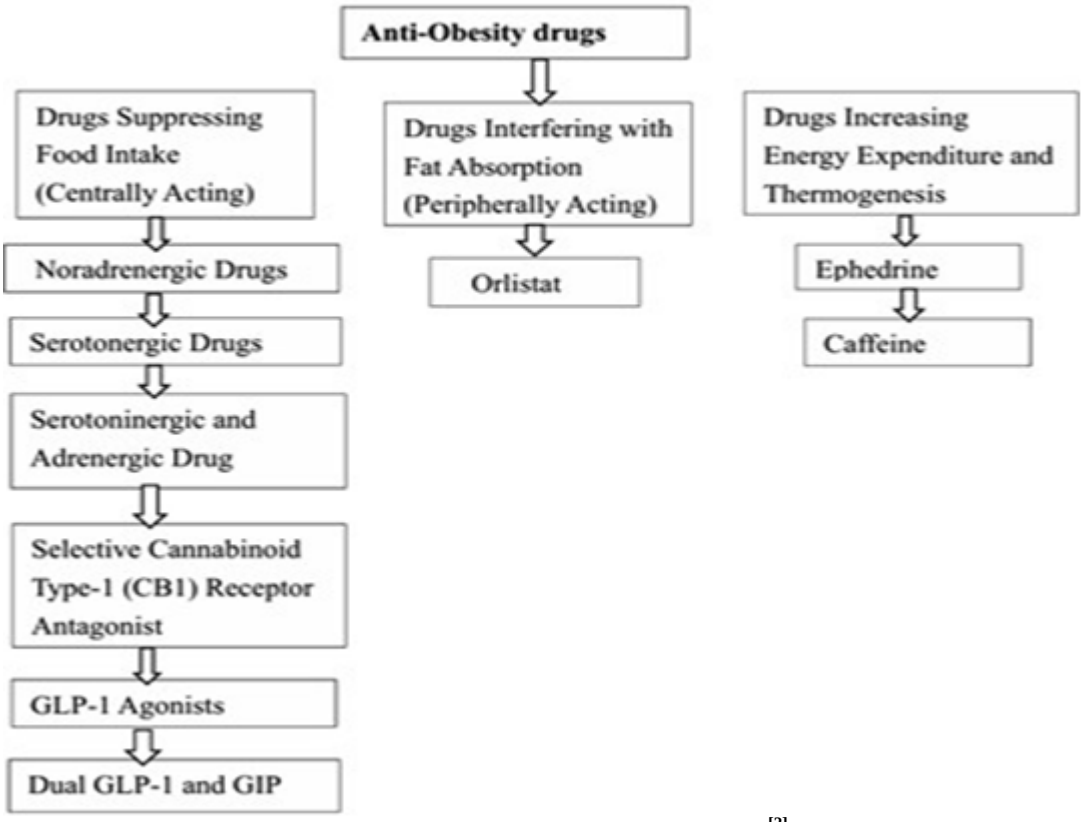

Fig. 1: Classification of Anti-obesity drug.[2]

1.4 Ratio of obesity

According to the survey,

Global trends: While obesity rates are rising for both men and women, the world obesity federation predicts that by the time of 2030, 1 in 5 women and 1 in 7 men will be living with obesity.

India: In India, the obesity rate increased from 1.2% in 1990 to 9.8% in 2022 for women and from 0.5% to 5.4% in 2022 for men.[3]

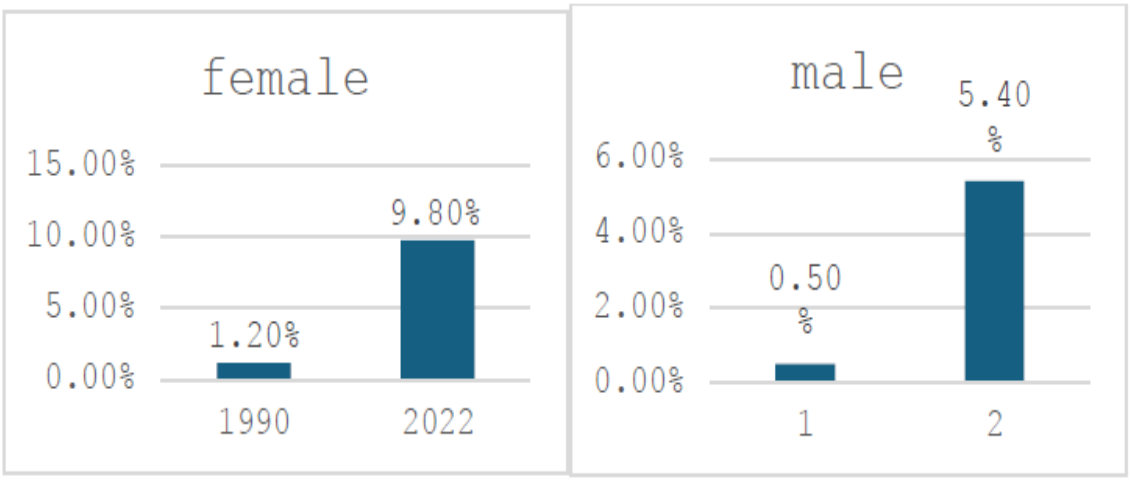

Fig. 2: Ratio of obesity.

2. Analytical method development

Expository strategy advancement is the method of planning and making specific explanatory strategies to analyse chemical tests precisely. It includes selecting the suitable explanatory strategies, optimizing exploratory conditions, and guaranteeing the strategy is appropriate for its intended purpose, such as distinguishing, isolating, evaluating, and characterizing chemical components in a substance, especially in pharmaceutical items.

It is guided by ICH Q 14 guidelines, involves a systematic approach to creating analytical procedures for

assessing the quality of drug substances and products. The goal is to establish procedures that are fit for purpose means they are suitable for measuring specific quality attributes with the required accuracy, precision, and specificity.[4]

2.1 ICH guidelines

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, or simply ICH, stands as a cornerstone in the realm of pharmaceutical development and regulation. The goal of ICH is to create global standardization for clinical trials. Each country or region's regulatory authorities (the FDA in the US, EMA in Europe, Health Canada, etc) create unique guidelines and requirements for conducting clinical research. However, many clinical trials aim to test on diverse populations, and eventually, distribute the resulting treatment across borders. By standardizing these requirements, ICH hopes to improve efficiency and safety in drug development worldwide.[5]

2.2 Different analytical methods to determine Anti-Obesity drugs

UV-Spectroscopy, HPLC, Bioanalytical method by, UPLC-MS, HPLC-MS, Gas chromatography, HPTLC.

2.2.1 UV Spectroscopy

Ultraviolet–visible (UV–Visible) spectrophotometry is primarily a quantitative analytical technique concerned with the absorption of near-UV (200–400 nm) or visible (400–800nm) radiation by chemical species in solution. These regions of the electromagnetic spectrum provide energy that gives rise to electronic transitions. The various colours of visible light and the complementary colours of solutions absorbing at particular wavelengths are shown. Because of the superimposition of vibrational and rotational transitions, the UV–visible spectrum of analytes in solution shows little fine structure.[6] Various analytical methods were developed by using UV Spectroscopy, Some of the latest methods are shown in **Table 1**

Table 1: Method development of anti-obesity drugs by UV spectroscopy.

Method	Drug	Model	Solvent	λ max	Dilution concentration	Reference
METHOD-1	Orlistat	t-60-uv-visible spectrophotometer with software UV win 5.0.10mm path length	Methanol	203nm Abs:0.165 -0.895	10-100µg/ml Precision: 1.562% Assay:99.85%	[7]
METHOD-2	Cetilistat	UV-Visible double beam spectrophotometer	n-hexane	320nm Abs: 0.189-0.955	20-100µg/ml Assay:101.1% Accuracy:96- 99% Precision:1.269%	[8]
METHOD-3	Cetilistat	A Systronic UV- Visible double beam spectrophotometer 2201 was used.	n-hexane and Ethanol (80:20)	321nm Abs: 0.567-0.960)	60-100µg/ml Assay: 97.73% Accuracy: 97-98%	[9]
METHOD-4	Febuxostat	A Systronic UV- Visible double beam spectrophotometer 2201 was used.	Methanol	312nm	1,2,3,4,5,6,10 µg/ml	[10]
METHOD-5	Imeglimin	laboholic-5131 UV-Visible spectrophotometer	Distilled water	247nm Abs: 0.097-0.488	2,4,6,8,10 µg/ml Precision:1.425% Assay:99.3%	[11]
METHOD-6	Semaglutide	UV 1800 double beam spectrophotometer with a pair of 10mm path lengths matched quartz cells were used.	Acetonitrile: Water(50:50)	293nm	10-50µg/ml	[12]
METHOD-7	Semaglutide	UV visible spectrophotometer T-60	Ethanol	288nm Abs: 0.142-0.791	5-30µg/ml Recovery:99.65% Assay:101.85%	[13]
METHOD-8	Semaglutide	UV visible spectrophotometer, Jasco v-60	Methanol	230nm	10-50µg/ml Recovery:90% Assay: 94.83%	[14]

2.2.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a highly effective tool in analytical chemistry, widely used for separating, identifying, and quantifying compounds in any liquid-dissolvable sample. It is considered one of the most precise methods for both qualitative and quantitative analysis of drug products.

The technique operates by injecting a sample solution into a column filled with a porous stationary phase, while

a liquid mobile phase pumped through the column under high pressure. Separation occurs due to differences in migration rates, which result from the varying partitioning of sample components between the stationary and mobile phases. Each component elutes at a distinct time based on its partitioning behaviour The primary goal of the HPLC method is to separate and quantify the main drug, reaction impurities, synthetic intermediates, and degradation products. HPLC has become one of the most advanced tools in analytical chemistry, capable of separating, identifying, and quantifying compounds in any liquid-dissolvable sample. It is widely recognized as one of the most reliable methods for both qualitative and quantitative analysis of drug products and for assessing their stability[15] Various analytical methods were developed by using HPLC. Some of the latest methods are shown in **Table 2**

Table 2: Method development of anti-obesity drugs by HPLC.

	HPLC-1	**HPLC-2**	**HPLC-3**	**HPLC-4**	**HPLC-5**	**HPLC-6**
Drug	Orlistat	Cetilistat	Cetilistat	Orlistat	Cetilistat	Orlistat
Model	A Waters HPLC model-2489	A Waters HPLC model.	Younglin HPLC system	Thermo-ultimate CAD. 300	HPLC-Jasco (Model/PU20801/UV- 2075-Plus, Jasco) with Borwin software	Precise isocratic reversed phase HPLC
Mobile phase	Acetonitrile	Acetonitrile, water, phosphoric acid (85:15:0.5)	Methanol, water, trifluoracetic acid (85:15:0.1)	Acetonitrile with water in the ratio (50:50)	Acetonitrile with phosphate buffer Ph 4.0(60:40)	Methanol, acetonitrile with 2%phosphoric acid (85:15:1)
Column	X Bridge C8 column, 130A 5µm	C18 Column 150x 4.6mm	C18 Column	C18 column 150 x 4.6mm	C18 Column 250x 4.6mm	Waters Spherisorb 5µm octadecyl-silica-2 250x 4.6mm column
Wavelength	202nm	205nm	222nm	205nm	228nm	215nm
Flow rate	1ml/min.	1ml/min.	1ml/min.	2ml/min.	1ml/min.	1ml/min.
Detector	UV detector	UV Detector	UV Detector (730D)	UV Detector	UV-visible detector	PDA Detector
Run time	10min	10min	7min	-	10min	-
LOD	1.76 µg/ml	0.054 µg/ml	1.48 µg/ml	39	1.961 µg/ml	0.06 µg/ml
LOQ	1.71 µg/ml	0.182 µg/ml	4.47 µg/ml	21	5.944 µg/ml	0.2 µg/ml
Retention time	4.7min	3.79min	3.643min	1.2min	2.73min	5.9min
Assay	-	-	-	99.3%	100.26%	99.83%
Volume of injection	10µl	-	20µl	-	10µl	20µl
References	[16]	[17]	[18]	[19]	[20]	[21]

2.2.3 HPLC –MS

High-performance liquid chromatography-mass spectrometry (HPLC- MS) combines the separation power of LC with the detection specificity of MS. It has become an indispensable analytical tool in the pharmaceutical industry due to its high throughput, sensitivity, and selectivity. The growing demand for drug discovery has challenged the applications of HPLC-MS in pharmaceutical analysis**.** Some of the latest methods are shown in Table 3.

Table 3: Determination of 12 anti-obesity drugs in human plasma by a 96-well protein precipitation plate using HPLC-MS.

	HPLC-MS/MS-1	**HPLC-MS/MS-2**	**HPLC-MS/MS-3**	**HPLC-MS/MS-4**	**HPLC-MS/MS-5**	**HPLC-MS/MS-6**
Drug	Amphetamine	Fenfluramine	Bupropion	Lovastatin	Fluoxetine	Sibutramine
model	HPLC- MS/MS Instrument	HPLC- MS/MS Instrument	HPLC- MS/MS Instrument	HPLC- MS/MS Instrument	HPLC- MS/MS Instrument	HPLC- MS/MS Instrument
Column	Agilent Poroshell 120 EC-C18	Agilent Poroshell 120 EC-C18	Agilent Poroshell 120 EC-C18	Agilent Poroshell 120 EC-C18	Agilent Poroshell 120 EC-C18	Agilent Poroshell 120 EC-C18
Mobile phase	0.1% formic acid with acetonitrile with	0.1% formic acid with acetonitrile with water	0.1% acetic acid with acetonitrile with water	0.1% formic acid with acetonitrile with water	0.1% formic acid with acetonitrile with water	0.1% acetic acid with acetonitrile with water

	water					
Detector	Electrospray ionization tandem mass spectrometry	Electrospray ionization tandem mass spectrometry	Electrospray ionization tandem mass spectrometry	Electrospray ionization tandem mass spectrometry	Electrospray ionization tandem mass spectrometry	Electrospray ionization tandem mass spectrometry
Volume of Injection	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
LOD	0.1-0.5ng/ml	0.1-0.5ng/ml	0.1-0.5ng/ml	0.1-0.5ng/ml	0.1-0.5ng/ml	0.1-0.5ng/ml
LOQ ng/ml	0.50	0.02	0.10	0.50	0.10	0.10
Retention time (mint)	1.766	6.624	6.473	9.736	7.120	7.248
Fragmentor voltage	14V	80V	80V	75V	80V	80V
Accuracy	96.23-100.46%	102.26-102.46%	99.48-103.65%	95.16-89.95%		87.56-91%94.56-105%
Run Time	20min	20min	20min	20min	20min	20min
References	[23]	[23]	[23]	[23]	[23]	[23]

2.2.3 Gas Chromatography – Mass Spectroscopy

GC-MS is often used to qualitatively and quantitatively determine organic compound purity and stability and to identify components in a mixture. GC-MS is commonly used in many disparate fields, including environmental chemistry for atmospheric, soil, and water research; forensic science for detection of drugs of abuse (or metabolites) and in arson fire debris analysis; food science for determination of food or beverage quality and authenticity; and in developing renewable fuels.[24][25] Some of the latest method is shown in **Table 4**

Table 4: Development and Validation of Gas Chromatography–Mass Spectrometry Method for Sibutramine in Dietary Supplements.

Drug	**Sibutramine**
Model	Bruker Scion 436-GC SQ MS, Bremen, Germany
Electron energy 70 eV	70 eV
Calibration curve concentration range	0.3 to 30 µg/mL
Column	A Bruker BR-5ms fused silica capillary column (0.25 µm film thickness and 15 m × 0.25 mm i.d.)
Detector	Mass spectrophotometer
Injection Tempreature	200-250°C
Run time	20min
Carrier gas Flow rate (Helium)	1ml/min
LOD	0.181µg/ml
LOQ	0.5488µg/ml
Retention time	6.71min
Injection volume	1µl (Splitless injection)
Run time	20min
Oven tempreature	The oven temperature was initially maintained at 60 ◦C for 1 min, then increased to 160 ◦C at 40 ◦C/min, and after that increased to 225 ◦C at 10 ◦C/min and held for 2 min
Transfer line temperature	300 ˚C
Reference	[26]

2.2.4 High Performance Thin layer Chromatography

High-performance thin-layer chromatography (HPTLC) is an advanced form of instrumental TLC, which does not only include the use of high-performance adsorbent layers (e.g. Silica gel with refined uniform particles, approximately 5µm in diameter, as compared to 12µm in TLC), but also adopted instrumentation.[22] Some of the latest methods are shown in Table 5

Table 5: Development and Validation of Sibutramine by using HPTLC.

Drug	**Sibutramine**
Column	C18 Perfectsil Target ODS-3
Mobile phase	Chloroform acetone and methanol (8.5:1:0.55)
Wavelength	223nm
Flow rate	1.0ml/min.
Injection volume	20µl

Run time	10min
LOD	92.23µg/ml
LOQ	279.92µg/ml
Recovery	99.08-107%
precision	~2.7%
Reference	[27]

CONCLUSION

The article has systematically reviewed the use of modern analytical tools, including UV- Visible Spectroscopy, HPLC, HPLC-MS/MS, GC-MS, and HPTLC, in the qualitative and quantitative assessment of various anti-obesity drugs such as Orlistat, Cetilistat, Semaglutide, and Sibutramine. Each technique offers distinct advantages depending on the drug molecule's physicochemical properties and the analytical goals—ranging from simple concentration measurement to intricate impurity profiling.

By aligning these techniques with ICH Q14 guidelines, the reviewed methods demonstrate high precision, accuracy, recovery, reproducibility, and compliance with global regulatory standards. The extensive comparative data, including parameters like wavelength, solvents, retention time, limit of detection (LOD), and limit of quantification (LOQ), confirm the suitability of each method for specific drug formulations and applications.

Moreover, the review reveals that HPLC and HPLC-MS/MS stand out for their precision and sensitivity, especially in complex biological matrices and stability studies. UV spectroscopy, while simpler and cost-effective, remains a vital initial screening tool. GC-MS proves instrumental in detecting volatile compounds and adulterants, particularly in dietary supplements, while HPTLC offers high throughput and rapid screening capability.

Importantly, as drug formulations become increasingly complex—especially in the field of anti-obesity therapeutics—the role of analytical method development becomes even more critical. Reliable analytical protocols are essential for optimizing laboratory efficiency, reducing time-to-market for new drugs, and ensuring patient safety.

In conclusion, this review emphasizes that the future of pharmaceutical analysis in obesity treatment hinges on the continued refinement of analytical methods, integration of advanced instrumentation, and harmonization with international regulatory frameworks. Investing in analytical science not only improves the efficacy of anti-obesity interventions but also contributes to the broader goals of global health and therapeutic innovation.

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