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ENVIRONMENTAL SCIENCE ARCHIVES
ISSN: 2583-5092
Volume V, Special Issue, 2026
Received: 1/18/2026 Accepted: 3/4/2026 Published: 3/8/2026
DOI: 10.5281/zenodo.18906883

RESEARCH PAPER OPEN ACCESS

A Simple and Validated Ce(IV)–Indigo Carmine Based Spectrophotometric Method for Pharmaceutical Analysis of a few commercial Drugs

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Abstract
A reliable and economical indirect spectrophotometric method was developed and validated for the quantitative determination of Imatinib Mesylate and Oseltamivir Phosphate in bulk drug samples and pharmaceutical dosage forms. The analytical procedure is based on the oxidation of the selected drugs with an accurately measured excess of ceric ammonium sulphate in a sulphuric acid medium. After completion of the oxidation reaction, the remaining unreacted oxidant was determined spectrophotometrically using Indigo Carmine as a chromogenic reagent. Experimental conditions affecting the analytical performance of the method, including acid concentration and reaction time, were carefully investigated and optimized to ensure maximum sensitivity and reproducibility. Under the optimized conditions, the method exhibited good adherence to Beer–Lambert's law over the selected concentration range. Method validation was carried out according to International Council for Harmonisation (ICH) guidelines, evaluating parameters such as accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness. Recovery studies demonstrated satisfactory accuracy and confirmed that commonly present pharmaceutical excipients did not interfere with the determination of the studied drugs. The proposed spectrophotometric procedure is simple, rapid, and cost-effective, and therefore can serve as a practical alternative to sophisticated instrumental techniques for routine quality control analysis of these pharmaceuticals.

Keywords: Indirect Spectrophotometry, Cerium (IV) Oxidation, Indigo Carmine Dye, Pharmaceutical Analysis, Method Validation.

Introduction

Imatinib mesylate (ITM) is a significant anticancer agent widely used in the management of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors, primarily because of its capability to inhibit tyrosine kinases such as Bcr-Abl (Pessetto et al., 2014). The drug was approved by the United States Food and Drug Administration (US-FDA) for the treatment of CML owing to its proven therapeutic effectiveness (Cohen et al., 2002). From a chemical perspective, imatinib mesylate is described as 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl] benzamide monomethane sulfonate (O'Neil, 2006). A number of analytical techniques have been reported for the determination of this drug in pharmaceutical formulations, including UV spectrophotometric methods (Alkharfy et al., 2013), high-performance liquid chromatography (HPLC) (Abdel Karim et al., 2014), charge-transfer spectrophotometric methods (Shah et al., 2014), and reverse-phase HPLC (RP-HPLC) procedures (Rele and Patil, 2019; Vivekanand, 2003).

Oseltamivir is an ester prodrug that belongs to the class of neuraminidase inhibitors (Moscona, 2005) and is widely prescribed for the treatment of influenza infections (McKimm-Breschkin, 2000). After metabolic activation in the body, its active form inhibits viral neuraminidase, thereby blocking viral replication and preventing the release of newly formed viral particles (Raut et al., 2010). Chemically, oseltamivir is designated as (3R,4R,5S)-4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester (O'Neil, 2006). Several analytical approaches have been described for its quantitative estimation, including UV spectrophotometric methods (Al-

Bagary et al., 2014), HPLC techniques (Malipatil et al., 2011), RP-HPLC methods (Youssef et al., 2013; Jain et al., 2017), HPTLC procedures (Green et al., 2008; Ravisankar et al., 2015), as well as colorimetric and chromatographic methods (Conners, 1982; Ashour and Aboudan, 2018).

Although numerous analytical techniques have been reported for the estimation of these drugs, simple oxidative spectrophotometric methods are comparatively limited. In the present work, an indirect spectrophotometric method is proposed based on the oxidation of the selected drugs using cerium (IV) sulphate (CAS) as an oxidizing agent, followed by the determination of the remaining oxidant using Indigo Carmine (IC) dye as the analytical reagent.

Structure of drugs

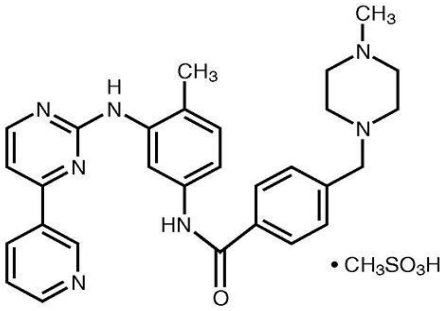

Fig. 1. Imatinib Mesylate

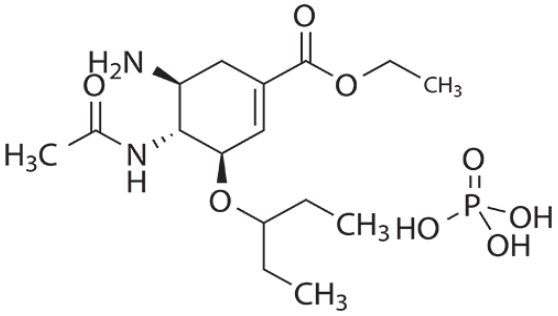

Fig. 2. Oseltamivir Phosphate

About the Method

Cerium (IV) is a powerful oxidizing agent comparable to commonly used oxidants such as $KMnO_4$ and $K_2Cr_2O_7$, and it has been extensively utilized in pharmaceutical analysis for the quantitative determination of drugs through oxidation-based reactions (Sastry and Rao, 1996; Basavaiah and Ramakrishna, 2003). In indirect spectrophotometric methods, a measured excess of Ce(IV) is allowed to react with the analyte, and the remaining unconsumed oxidant is subsequently determined using appropriate chromogenic dyes including Indigo Carmine, Methyl Orange, Safranin-O, and Xylene Cyanol. Among these reagents, Indigo Carmine has been reported to be particularly suitable for the estimation of residual Ce(IV) at 610 nm, mainly because of the high oxidation potential of Ce(IV) ($E° = 1.44$ V) (Basavaiah and Prameela, 2003; Sastry et al., 1998).

Instrumentation

UV–Visible absorption spectra were measured using ELICO 210 double-beam, Thermo Nicolet 1000, and ELICO 159 single-beam spectrophotometers, each fitted with 10 mm quartz cuvettes. Accurate weighing of samples was performed using a Dhona 200 single-pan electronic analytical balance.

All chemicals and reagents used in the study were of analytical reagent (AR) grade, and distilled water was used throughout the experimental work. A standard Cerium (IV) solution was prepared by dissolving 750 mg of cerium (IV) sulphate ($CeSO_4 \cdot 2H_2O$, 99.9%, Merck, Mumbai) in 2 N sulphuric acid with gentle warming. The resulting solution was filtered through glass wool and then diluted to 250 mL with the same acid medium. The prepared solution was standardized using ferrous ammonium sulphate as the titrant with ferroin indicator. Subsequently, it was further diluted with 2 N H_2SO_4 to obtain a working solution having a concentration of 4.0×10^{-3} M (0.25%).

Indigo Carmine (IC) dye: A stock solution of Indigo Carmine was prepared by dissolving 112 mg of the dye (Sigma-Aldrich, 90% purity) in bidistilled water and making up the volume to 100 mL, giving a concentration of 1000 µg mL^{-1}. A working solution of 200 µg mL^{-1} was subsequently prepared by suitable dilution of the stock solution with bidistilled water.

Sulphuric acid: 2 M sulphuric acid solution was prepared by suitably diluting concentrated H_2SO_4 (Merck, Mumbai, India; specific gravity 1.84, 98%) with distilled water. Preparation of drug solution: A standard drug solution (200 µg mL^{-1}) was prepared by accurately weighing 20 mg of each drug, dissolving it in a suitable solvent, and diluting the solution to 100 mL in a volumetric flask. The stock solutions of ITM and OSP were subsequently diluted with the same solvent to obtain the required working concentrations.

Procedure

Aliquots containing 3–21 µg mL^{-1} of ITM and 3.5–24.5 µg mL^{-1} of OSP were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of ceric ammonium sulphate (CAS) and 1 mL of 2 M H_2SO_4 were added and the contents were mixed thoroughly. The mixture was allowed to react for 15 min, after which 1 mL of 0.02% Indigo

Carmine dye was added. The solutions were then made up to the mark with double-distilled water, and the absorbance was measured at 610 nm against a reagent blank.

Assay of Pure Drug Sample

Pure drug solutions within the Beer's law range (3–21 µg mL^{-1} for ITM and 3.5–24.5 µg mL^{-1} for OSP) were used to evaluate the accuracy and precision of the proposed method. Each solution was treated with 1 mL of cerium (IV) solution and 1 mL of 2 M H_2SO_4, and the remaining oxidant was subsequently estimated using Indigo Carmine dye according to the procedure described earlier. Calibration curves were prepared from six replicate measurements, considering responses within 95–105% of the average value.To further assess the accuracy and precision of the developed method, pure sample solutions containing the drugs within the Beer's law concentration limits were selected, namely 3–21 µg mL^{-1} for ITM and 3.5–24.5 µg mL^{-1} for OSP.

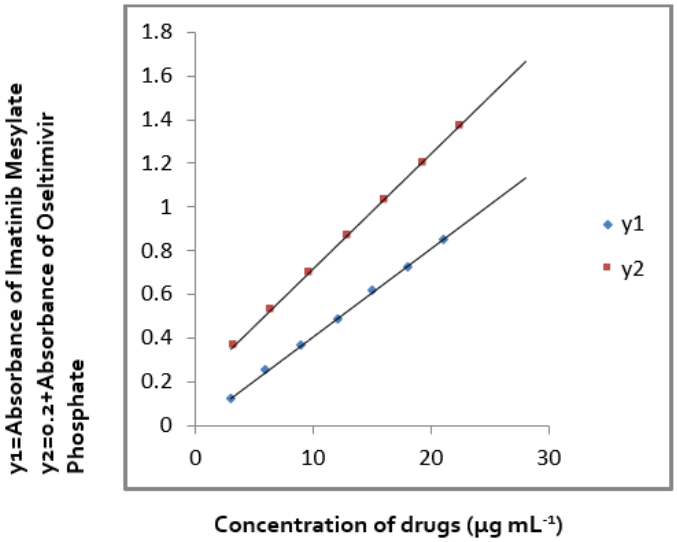

Fig. 3. Calibration curves of drugs ITM and OSP

Procedure For Assay of Pure Drug

Sample solutions within the Beer's law range were analyzed, and recovery studies were carried out to assess the accuracy and precision of the method. The results (Table 2) demonstrated excellent recovery values, with %RSD less than 2%, confirming the reliability and reproducibility of the proposed method.

Procedure For Analysis of Tablets

Imatinib Mesylate

For pharmaceutical analysis, tablets (Veenat, 100 mg) were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Imatinib mesylate was transferred into a 100 mL volumetric flask, dissolved in double-distilled water, and filtered through Whatman No. 42 filter paper. The filtrate was then diluted to the mark with the same solvent to obtain the stock solution, which was further diluted appropriately to prepare the required working solutions.

Oseltamivir Phosphate

Two capsules (Tamiflu, 75 mg) were opened and their contents were carefully collected. A portion equivalent to 10 mg of Oseltamivir Phosphate was transferred into a 100 mL volumetric flask, dissolved in double-distilled water, and filtered through Whatman No. 42 filter paper. The filtrate was then diluted to the mark with the same solvent to obtain the stock solution, which was subsequently diluted appropriately to prepare the required working standard solutions.

Method of Validation

Each method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), selectivity, and ruggedness. Precision was evaluated by six replicate analyses, while accuracy was assessed through percent recovery and %RSD values (<2%), confirming the reliability of the method (Table 2).

The LOD, representing the lowest detectable concentration, was calculated using:

$$LOD = 3.3\, S_a/S$$

The LOQ, indicating the lowest quantifiable concentration, was calculated as:

$$LOQ = 10\, S_a/S$$

where S_a is the standard deviation of the intercept (n = 6) and S is the slope of the calibration curve.

The linearity range of the calibration curves followed Beer's law limits (Fig. 3). Selectivity was confirmed through recovery studies in the presence of common excipients, while ruggedness was evaluated using different analysts and instruments, showing no significant variation in results.

Factors Effecting Absorbance and Selection of Acid

Selection of acid: The effect of different acids (H_2SO_4, H_3PO_4, and CH_3COOH) was studied to obtain maximum redox reaction efficiency. Sulphuric acid was found to be the most suitable medium for Ce(IV) oxidation.

Effect of acid concentration: Various concentrations and volumes of H_2SO_4 were examined, and maximum absorbance was obtained using 1 mL of 2 N H_2SO_4, which was selected for further analysis.

Effect of time: The influence of reaction time (2.5–20 min) was investigated, and maximum stable absorbance was achieved after 15 minutes, which was considered optimal.

Analysis of Pharmaceuticals

To assess the applicability of the proposed method, pharmaceutical tablet solutions within Beer's law limits were analyzed. Each sample was examined in six replicates to evaluate precision, while accuracy was determined from percent recovery and %RSD values. The results showed excellent recovery with %RSD less than 2% (Table 3), confirming the suitability of the method for routine pharmaceutical analysis.

Results and Discussion

The proposed indirect spectrophotometric method is based on the strong oxidizing nature of cerium (IV) sulphate, which readily oxidizes pharmaceutical drug molecules in an acidic medium (Basavaiah and Charan, 2002; Sastry and Rao, 1996). In this procedure, a known excess of Ce(IV) was allowed to react with the drug solution under optimized conditions. During the reaction, part of the oxidant was reduced to Ce(III), while the remaining unreacted Ce(IV) was subsequently determined using Indigo Carmine (IC) dye.

The residual Ce(IV) oxidizes Indigo Carmine, leading to a decrease in the dye concentration that can be monitored spectrophotometrically at 610 nm. As the drug concentration increases, a greater amount of Ce(IV) is consumed during oxidation, leaving less oxidant available for dye bleaching. Consequently, a larger amount of unoxidized Indigo Carmine remains in solution, resulting in increased absorbance. This behavior establishes a linear relationship between absorbance and drug concentration within Beer's law limits.

Optimization studies showed that 1 mL of 2 N sulphuric acid provided maximum reaction efficiency and stable absorbance values, and therefore this condition was selected for all measurements.
The reactions involved in the method can be represented as:

+ Ce(IV) (excess) → Oxidized drug + Ce(III) + unreacted Ce(IV)

Unreacted Ce(IV) + Indigo Carmine → Oxidized dye + residual Indigo Carmine

The absorbance of the remaining Indigo Carmine was measured at λmax = 610 nm, which forms the analytical basis for the quantitative determination of the drugs.

Scheme 1: Reaction scheme for the indirect spectrophotometric determination of drugs using cerium (IV) sulphate as the oxidizing agent.

Analytical Data

A good linear relationship was observed between the absorbance at λmax and drug concentration within the studied range. Sensitivity parameters, including Sandell's sensitivity, limit of detection (LOD), and limit of quantification (LOQ), were calculated in accordance with ICH guidelines (ICH, 1994; ICH, 1996) and are presented in Table 1, indicating the high sensitivity of the method. Regression analysis of the Beer's law data using the least-squares method yielded the slope (b), intercept (a), and correlation coefficient (r), which are also summarized in Table 1.

Accuracy and precision were evaluated by analyzing pure drug solutions at six concentration levels within working limits. The relative error (%) and %RSD values summarized in Table 2 indicate the high accuracy and precision of the proposed methods.

Robustness and Ruggedness

Robustness of the method was evaluated by introducing slight variations in acid volume and reaction time (10 ± 2 min). Ruggedness was examined by conducting the analysis with different analysts and spectrophotometers, and no significant variation in the results was observed.

Table 1. Analytical and Regression parameters of Spectrophotometric Method

Name of drug Property	ITM	OSP
λ_{max}, nm	610	610
Beer's law limits (µg mL^{-1})	3.1-21.0	3.4-24.5
Molar absorptivity	$2.073x10^4$	$1.392x10^4$
Sandell's sensitivity (µg cm^{-2})	0.0258	0.0306
Variance $(S_a)^2$	0.000041	0.0000022
Limit of detection µg mL^{-1}	0.18082	0.17131
Limit of quantification µg mL^{-1}	0.5482	0.5191
Regression equation, Y**		
Intercept, (a)	0.00729	0.00289
Slope, (b)	0.03739	0.03118
Correlation coefficient, (r)	0.9994	0.9997
Standard deviation of intercept (S_a)	0.0021	0.0017
Standard deviation of slope (S_b)	0.00149	0.00208

**Limit of determination is defined as the weight in µg mL^{-1} of solution corresponding to an absorbance of A = 0.001, measured in a cuvette with a path length of 1 cm and a cross-sectional area of 1 cm^2.*

***Regression equation: Y = a + bX, where Y represents the absorbance and X denotes the drug concentration in µg mL^{-1}.*

Table 2. Determination of accuracy and precision of the methods on pure drug Samples

Drug	Taken (µg/ml)	Found (µg/ml)	error (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
ITM	3.0 6.0 9.0	2.99 6.01 8.98	0.33 0.17 0.22	99.68 100.16 99.77	0.0851	99.76 ±0.0848
OSP	3.5 7.0 10.5	3.49 7.01 10.51	0.28 0.14 0.09	99.70 100.15 100.10	0.2348	99.97 ±0.2347

Application to Formulations

The proposed spectrophotometric methods were successfully applied for the quantitative determination of the selected drugs in commercial tablet formulations. The analytical results obtained (Table 3) demonstrated accurate and reliable estimation of drug content, indicating that commonly used pharmaceutical excipients present in the dosage forms did not interfere with the analysis. The assay results were compared with those obtained from previously reported validated methods (Patil et al., 2013; Al-Bagary et al., 2014; Ravisankar et al., 2015) and showed close agreement with the labelled claim values as well as with literature data.

Table 3. Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by the standard addition method

Tablets	Drug in tablet µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	Error (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Proposed method Mean± SD
Veenat (ITM)	0.50 0.50 0.50 3.0 6.0 9.0	3.0 6.0 9.0 0.0 0.0 0.0	3.51 6.48 9.52 3.00 5.99 9.01	0.28 0.31 0.21 0.00 0.16 0.11	100.29 99.68 100.20 100.01 99.82 100.12	0.22549	100.51 ±0.549 (n=3)	100.022±0.2255
Oseltimivir Phosphate (OSP)	0.50 0.50 0.50 3.5 7.0 10.5	3.5 7.0 10.5 0.0 0.0 0.0	3.99 7.52 11.0 3.49 7.01 10.52	0.25 0.27 0.00 0.28 0.14 0.19	99.76 100.26 100.01 99.70 100.15 100.18	0.21639	100.31 ±0.11 (n=6)	100.02 ±0.2163

Further statistical evaluation of the results was performed using Student's t-test to assess accuracy and the F-test to evaluate precision (Miller and Miller, 2010). The calculated values indicated no significant difference between the proposed methods and the reported methods at the 95% confidence level, confirming the reliability and analytical performance of the developed procedures.

The accuracy and applicability of the methods were further confirmed through recovery studies using the standard addition technique. Known quantities of pure drug were added to pre-analysed tablet samples at three concentration levels (50%, 100%, and 150% of the nominal drug content), and the total drug content was

subsequently determined. Each experiment was carried out in six replicates, and the percentage recovery values obtained were within acceptable analytical limits. These findings indicate the absence of interference from common excipients and demonstrate that the proposed methods are suitable for routine pharmaceutical quality control analysis.

Table 4. F-test and t-test values

	Veenat (ITM)	**Oseltamivir Phosphate (OSP)**
F-test*	0.303 (4.7571)	0.012 (4.2839)
t-test**	0.9811 (3.182)	1.1952 (2.447)

*t- test and **F-test values from literature.

Conclusion

The present study reports the successful development of a simple, sensitive, selective, accurate, and rapid spectrophotometric method for the determination of the selected drugs in pharmaceutical formulations using cerium (IV) sulphate as an effective oxidizing agent. The proposed method exhibited excellent analytical performance without interference from commonly used additives and excipients present in dosage forms. Owing to its simplicity, reliability, and cost-effectiveness, the method can be conveniently applied for the analysis of both pure drug samples and pharmaceutical formulations. Hence, the developed procedure provides a suitable alternative to previously reported analytical methods for routine drug determination.

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Author Contributions
RB conceived the concept, wrote and approved the manuscript.

Acknowledgements
The authors thank Prof. M. Sayaji Rao (Retd.), Department of Chemistry, University College of Science, Osmania University, for guidance.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Competing interest
The author declares no competing interests.

Ethics approval
Not applicable.

Citation: Balusani R (2026) A Simple and Validated Ce(IV)–Indigo Carmine Based Spectrophotometric Method for Pharmaceutical Analysis of a few commercial Drugs. Environmental Science Archives 5 (Special Issue): 152-158.