Validation of An Efficient 2D-HPLC Method for The Determination of Pentazocine

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Abstract: *Pentazocine is an opioid analgesic used to treat moderate to severe pains. The real analysis for the pharmaceutical products containing pentazocine is basically to ensure the correct dose and patient safety. This study developed and validated an improved high-performance liquid chromatography (HPLC) method in the selective and accurate quantification of pentazocine. Two-dimensional (2D) HPLC technique is employed to enhance the resolution and selectivity compared to conventional HPLC methods. The 2D-HPLC instrumentation consists of two C18 columns coupling with a switching valve to capture fractions from the first column, which is analyzed in the second column. The mobile phase was optimized to 45% acetonitrile and 55% water with 0.1% phosphoric acid. The method was validated in the International Conference on Harmonization guidelines and shows excellent linearity (R^2=0.998), limit of detection of 1.58 µg/L, accuracy of 97.70–102.50%, and precision by relative standard deviation (RSD) of 1.02–4.20%. Selectivity was verified in resolving pentazocine from paracetamol, caffeine, ibuprofen, and oxycodone in laboratory mixtures. The utility of the 2D-HPLC method was demonstrated by accurate quantification of pentazocine in pharmaceutical injections and tablets unaffected in excipients. This research provides a rich validating technique to enhance the quality control testing of pharmaceuticals containing pentazocine.*

Keywords: *pentazocine; quantification; selectivity; validation; 2D-HPLC*

■ INTRODUCTION

Pentazocine ($C_{19}H_{27}NO$) is an organic compound (Fig. 1), which is categorized as opioid pain medication [1]. It is a common alternative to other opioid analgesics such as morphine, heroin, and fentanyl, as its analgesic, anti-allergic, and stimulant effects [2]. It is primarily used to relieve moderate to severe pain and alleviate pain caused by surgeries and acute injuries [3]. Opioid analgesics are a class of drugs used commonly in medicine that work by acting in opioid CNS receptors and on various regions of the body [4]. While pentazocine has analgesic properties that are similar to those of other opioid analgesics, it has unique anti-opioid and adrenergic stimulant properties that make it useful for the treatment of chronic pain, especially pain associated with serious illnesses such as cancer [5].

Active pharmaceutical ingredient (API) analysis

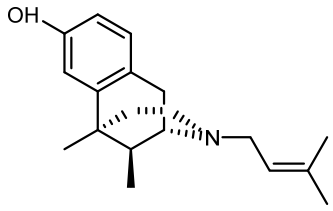

Fig 1. The pentazocine structure

helps to verify the quality of the drug and establish that they contain accurate and consistent amounts for required technical specifications, ensuring patient safety and treatment efficacy [6]. It helps to adhere to health regulations in the pharmaceutical industry requiring the check of the concentration of API and its tolerability [7].

Pentazocine has been quantified in various pharmaceutical samples by different analytical techniques [8-10]. Analytical techniques used in the quantification of pentazocine, including voltammetric

methods [11-12], UV-vis spectroscopy [13-15] and gas chromatography (GC) [16-17] and high-performance liquid chromatography (HPLC) [18-20].

Our study hypothesizes that the application of novel two-dimensional (2D) HPLC method can significantly enhance the analysis of pentazocine within pharmaceutical products [21-22]. The objective is to raise conditions for accuracy and establish strong quality control standards for the analysis of pentazocine. This involves the employment of sequential separation technology in HPLC, allows for improved compound separation and minimizes interference from coexisting compounds [23]. The significance of presenting our study lies in its potential to deal with the limitations of current HPLC techniques [21], such as poor resolution and interference problems with complex samples. Developing and validating an improved 2D-HPLC method [22] in this research seeks to provide a more selective and sensitive analytical approach to assess the pentazocine drug in the management of pain.

The implications of this research are important in the pharmaceutical field of analysis. The 2D-HPLC method [23] characterized by high precision and sensitivity promises more accurate identification and quantification of the pentazocine to ensure the quality and safety of the pharmaceutical products. Additionally, with aligning to International Conference on Harmonization (ICH) recommendations for method validation our study establishes an example for future analytical methodologies ensuring that the pharmaceutical products meet the higher standards of efficacy and safety.

■ EXPERIMENTAL SECTION

Materials

Pentazocine, paracetamol, caffeine, ibuprofen, oxycodone, pentazocine hydrochloride, acetaminophen, and ZORBAX SB-C18 resin were purchased from Sigma Aldrich (St. Louis, MO). Acetonitrile, phosphoric acid, and methanol were purchased from Merck.

Instrumentation

The compounds were measured using a 2D-HPLC system from Shimadzu Corporation, comprising an L-2455 diode array detector, an L-2200 quad pump, and an L-2350 column temperature controller. The first dimension used an RP8 HIBAR analytical column from Merck. The pH measurements were conducted with an Orion Research Model 601 digital pH meter and Ingold U455 electrode. The second dimension employed an Agilent "1290" Infinity 2D-LC system, with column selection and mobile phases tailored to specific requirements. Heart-cutting utilized twelve 40 µL loops in valve systems for multiple cuts, and an Agilent 6550 QTOF was incorporated for MS when necessary.

Procedure

Evaluation of method performance characteristics

The method validation was determined for pentazocine adhered to ICH guidelines, focusing on selectivity, linearity, sensitivity, and accuracy. Selectivity was confirmed by consistent retention times by standard solutions and blank matrices and samples ensuring minimal interference. Strong linearity was demonstrated with an R^2 value over 0.99. Sensitivity was evaluated using low limit of detection (LOD) and quantification (LOQ) enabling detection of trace level. Accuracy assessed during calibration curve method with five replicates by standard concentrations showed 98–102% recoveries meeting the criteria of acceptance. These results declare the effectiveness of method to accurately and precisely measure pentazocine. Precision refers to the nearness of repeated measurement and was evaluated with relative standard deviation (RSD) of repeatability and experiments of recovery. The RSD of repeatability show < 5% and the RSD of recovery show 98–102%, thereby meeting the criteria of stated precision.

Standard solution and calibration plot

To prepare a 1 mg/L standard pentazocine solution, 1.02 g of pure pentazocine was dissolved in 1,000 mL of bi-distilled water, ensuring homogeneity. Dilute 0.1 mL of this solution to 100 mL for a final concentration of 1 mg/L. For calibration solutions ranging from 10.0 to 150.0 µg/L, dilute specific volumes (1.0 to 15.0 mL) of the 1 mg/L solution into 100 mL volumetric flasks, and fill to volume with bi-distilled water. This process yields concentrations of 10.0 to

150.0 µg/L, following precise measurement and thorough mixing to ensure consistent sample preparation.

Sample solution

Three pharmaceutical samples were analyzed pentazocine injection (30.0 mg/1 mL), Talwin ampule (30.0 mg/1 mL), and pentazocine hydrochloride with acetaminophen tablets (25.0 mg/650.0 mg). For liquid samples, 1.0 mL was diluted in a 100 mL flask using 0.1 mL of the sample solution. For tablets, 0.780 g of powdered sample was dissolved in a 1 L flask, then 0.1 mL of this was transferred to a 100 mL flask and mixed with 50.0 mL of a blank solution, followed by stirring and sonochemical treatment for 5 min. The final concentration of pentazocine in all samples was adjusted to 30.0 µg/L.

Chromatography conditions

For 2D-HPLC analysis, a 20 µL sample was injected using a flow rate (FR) of 1 mL/min. Two C18 chromatography columns were employed: the first (250 × 4.6 mm, 5 µm) and the second (100 × 2.1 mm, 5 µm) packed with ZORBAX SB-C18 resin. The mobile phase for the first column comprised 45% acetonitrile, 55% water, and 0.1% phosphoric acid. Detection used a UV detector at 254 nm. A valve between the 1D detector and the 2D column captured 1D effluent fractions, transferring them to the 2D column via a sampling loop that alternated between collection and injection, as shown in Fig. 2.

■ RESULTS AND DISCUSSION

Optimization of Experimental Conditions

Flow rate FR adjustment

Three pharmaceutical samples, pentazocine injection, Talwin ampule (both 30 mg/mL), and pentazocine hydrochloride-acetaminophen tablets (25/650 mg), were analyzed. Liquid samples were diluted from 1 to 100 mL, and tablet samples, weighing 0.78 g, were dissolved and similarly diluted to 100 mL, including a 50 mL blank solution addition. The samples underwent stirring and sonochemical treatment, resulting in a standardized Pentazocine concentration of 30 µg/L for all.

Lower flow rates (FRs) caused increased analyte interference, while above 1.2 mL/min, recovery declined due to poor separation (Fig. 3). An optimal FR of 1.0 mL/min, within the stable 0.8–1.2 mL/min range, was selected for all experiments, with adjustments for varying sample matrices.

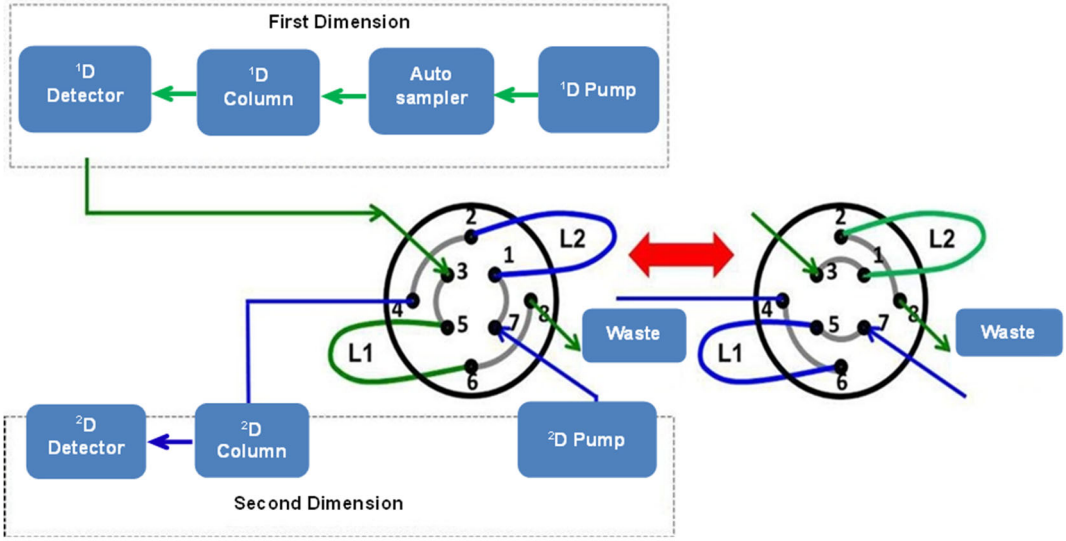

Fig 2. A diagrammatic illustration of a typical 2D-HPLC apparatus underscoring the criticality of the valve positioned between the 1D detector and the 2D column that seizes fractions of the 1D effluent and infuses them into the 2D column. The red arrow indicates that shifting the valve's position facilitates the role reversal of each sampling loop between gathering the 1D effluent and injecting it into the 2D column [24]

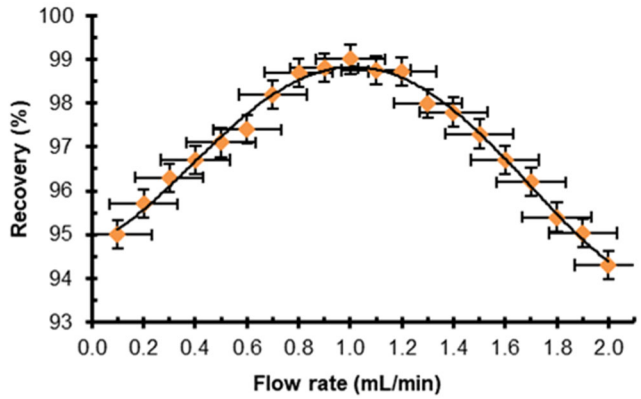

Fig 3. The effect of flow rate on the recovery of pentazocine using the proposed 2D-HPLC method, 30 µg/L of pentazocine and injected volume of 40 µL

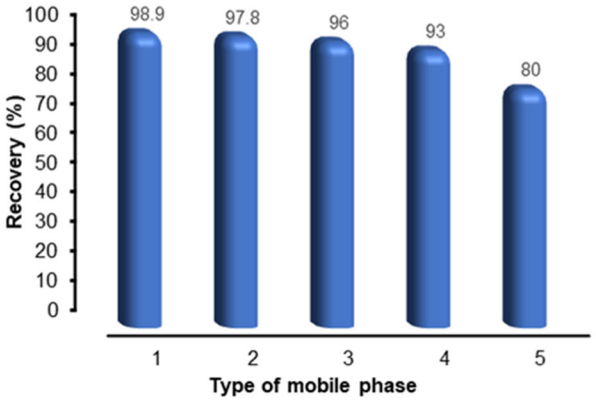

Fig 4. The effect of mobile phase type of pentazocine by using the proposed 2D-HPLC method, 30 µg/L of pentazocine and injected volume of 40 µL

Examining the impact of mobile phase type in the study

The study investigated the impact of mobile phase composition on HPLC analysis of pentazocine using five different phases, with a constant FR of 1.0 mL/min, column temperature at 25 °C, and a predetermined detection wavelength. The tested mobile phases were: (1) 45% acetonitrile, 55% water with 0.1% phosphoric acid, (2) 50% acetonitrile, 50% water, (3) 30% methanol, 70% buffer solution (4) 40% methanol, 60% saline solution, and (5) 35% tetrahydrofuran, 65% water.

The phase with 45% acetonitrile and 55% water with 0.1% phosphoric acid appeared the higher pentazocine recovery and best chromatographic performance, including good separation and good peak resolution (Fig. 4). Other phases exhibit the lowest recovery and inadequate separation from the varying elution strengths of methanol and water and the influence of buffers and salts. The acetonitrile-based phase attracts attention in optimal chromatographic conditions, offering superior sensitivity signal strength, and accuracy as pentazocine chemical interaction is different in each mobile phase's solvent strength polarity pH, and additives.

The optimization of the 2D-HPLC method for the pentazocine analysis involved systematic evaluation of the chromatographic state. A C18 reverse phase column of strong retention to pentazocine, was used in both dimensions a 250 × 4.6 mm column of 5 µm particles to the first and a 100 × 2.1 mm narrow bore column to the second. Optimal separation and recovery were done by using a mobile phase of 45% acetonitrile 55% water, and 0.1% phosphoric acid. The FR was put at 1.0 mL/min, balancing analysis time and resolution, and then the detection wavelength was optimized to 254 nm to maximum sensitivity. The injection volume was 20 µL, and the column temperature was maintained at 25 °C as high or low temperatures did not improve performance significantly.

The clear 2D-HPLC conditions include specific column sizes and packing, a carefully chosen mobile phase, a calculated FR, optimal detection wavelength appropriate injection volume, and control column temperature. This tailored approach ensures a sensitive and accurate method for pentazocine analysis applicable for all further experiments with this validated methodology.

The Validation

The accuracy was verified using the ICH guidelines [25-26] with 2D-HPLC (Table 1). The method's selectivity was evaluated by comparing the retention times of pentazocine in standard solutions, blank samples, and other samples, and no significant interference in retention times was observed, indicating a good selectivity of the method, Fig. 5. The 2D-HPLC method demonstrates linearity for pentazocine between 10.0 to 150.0 µg/L with an R^2 of 0.998, indicating excellent linearity Fig. 6. The LOD was established

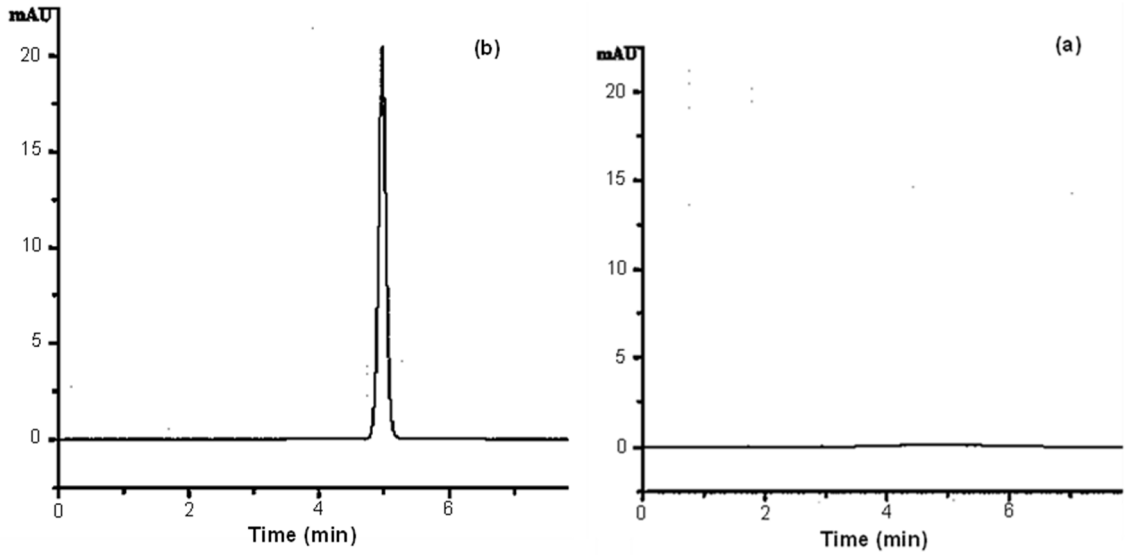

Fig 5. 2D-HPLC chromatograms: (a) a blank sample, and (b) a 30 µg/L pentazocine standard, with a 5 min retention time, injected volume of 40 µL. The elution used acetonitrile, water, and phosphoric acid (45:55:0.1) at a 1.0 mL/min FR and 25 °C temperature

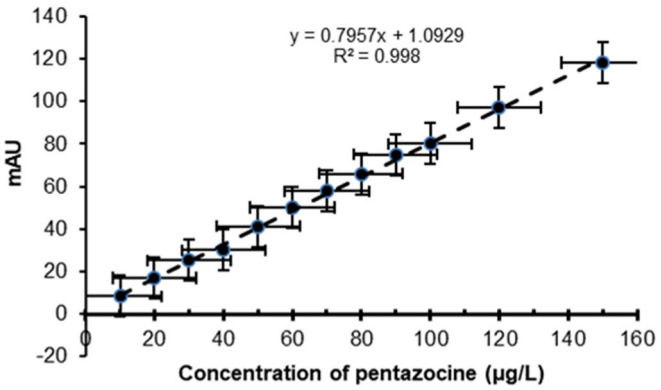

Fig 6. Calibration curve of pentazocine at concentrations ranging from 10–120 µg/L versus D2 detector response. The eluting agent was a combination of acetonitrile, water, and phosphoric acid in a ratio of 45:55:0.1, with an FR of 1.0 mL/min at 25 °C

to 1.58 µg/L lower than the lowest standard concentration of 10 µg/L significantly demonstrates the high sensitivity method to detecting trace levels of the pentazocine. Precision and reliability were declared by analyzing the replicates and evaluating sample recovery, with results falling within the 97.70–102.52% range acceptance. This underlines the effectiveness of the method in accurate quantification, even in concentrations much lower than the standard range, making it suitable for trace analysis.

Table 1. Precision and accuracy evaluation for pentazocine determination

Taken µg/L (n = 5)	Found µg/L $\bar{x} \pm S$	R (%)	RSD (%)
10.0	9.77 ± 0.41	97.70	4.20
20.0	19.64 ± 0.80	98.20	4.08
30.0	29.67 ± 1.15	98.90	3.87
40.0	39.64 ± 1.39	99.10	3.51
50.0	49.70 ± 1.60	99.40	3.23
60.0	59.82 ± 1.65	99.70	2.76
70.0	71.76 ± 1.69	102.50	2.36
80.0	80.08 ± 1.78	100.10	2.22
90.0	89.46 ± 1.68	99.40	1.88
100.0	99.10 ± 1.73	99.10	1.75
120.0	118.08 ± 1.77	98.40	1.50
150.0	147.30 ± 1.50	98.20	1.02

The method accuracy was determined by calculating RSD within a single day (intraday) by applying five replicates and over five consecutive days (interday) under the same experimental conditions. The results are summarized in Table 2. The intraday RSD was from 1.02–4.20%, while the interday RSD was from 1.27–4.82%. The method exhibited high precision and accuracy, with RSD ranging from 1.02 to 4.82% for both intraday and interday measurements, meeting ICH

Table 2. Interday and intraday RSD% results for the determination of 30 µg/L Using 2D-HPLC (n = 5)

Taken µg/L	RSD% (intraday)	RSD% (interday)
10.00	4.20	4.82
20.00	4.08	4.53
30.00	3.87	4.11
40.00	3.51	3.74
50.00	3.23	3.54
60.00	2.76	3.08
70.00	2.36	2.74
80.00	2.22	2.64
90.00	1.88	2.30
100.00	1.75	1.97
120.00	1.50	1.57
150.00	1.02	1.27

acceptance criteria. The comprehensive evaluation of the 2D-HPLC method confirmed its validity and suitability for pentazocine analysis, successfully meeting all criteria, including selectivity, system suitability, linearity, LOD, LOQ, accuracy, and precision.

Selective Determination of Pentazocine using 2D-HPLC

To assess the selectivity of the method for pentazocine detection, three laboratory drug mixtures were prepared, simulating common pharmaceutical combinations [27]. One such mixture included equal parts of pentazocine, paracetamol, and caffeine at 30 µg/L. Their simultaneous analysis was performed using an HPTLC normal phase system with silica gel 60F254 and a solvent mix of acetone, methanol, and water, measured at 254 nm with a 20 mL/min FR, as illustrated in (Fig. 7).

The study revealed that the retention times of caffeine, paracetamol, and pentazocine converged when analyzed using a reference method [28], leading to reduced separation efficiency due to peak overlapping. However, the researched technique demonstrated clear separation and no peak overlapping, enabling efficient simultaneous analysis of these compounds (Fig. 7(b)). In another mixture containing ibuprofen and pentazocine, the reference method resulted in long retention times and asymmetric peaks. Conversely, the researched method showed shorter retention times, symmetric and narrower peaks, albeit with reduced ibuprofen sensitivity due to the 254 nm measurement wavelength favoring pentazocine detection. These findings highlight the method's capability for effective analysis of pentazocine in complex mixtures (Fig. 8(b)).

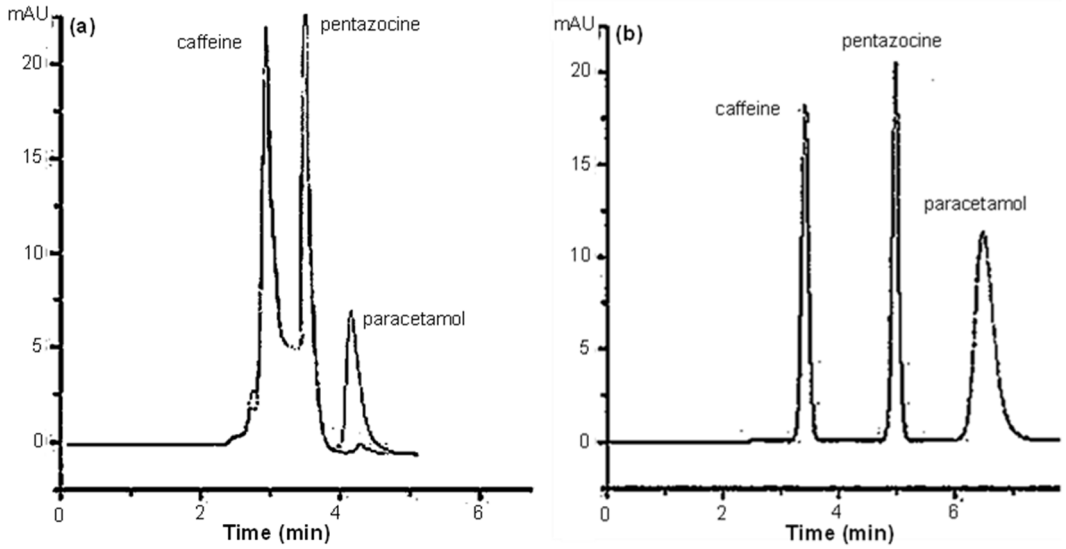

Fig 7. HPLC chromatograms for a mixture of pentazocine, paracetamol, and caffeine with 30 µg/L for each, where (a) according to a reference method HPTLC: acetone, methanol, and water (7:2.5:0.5, v/v), FR of 20 mL/min, and (b) according to a 2D-HPLC studied method: acetonitrile, water, and phosphoric acid in a ratio of 45:55:0.1, with an FR of 1.0 mL/min

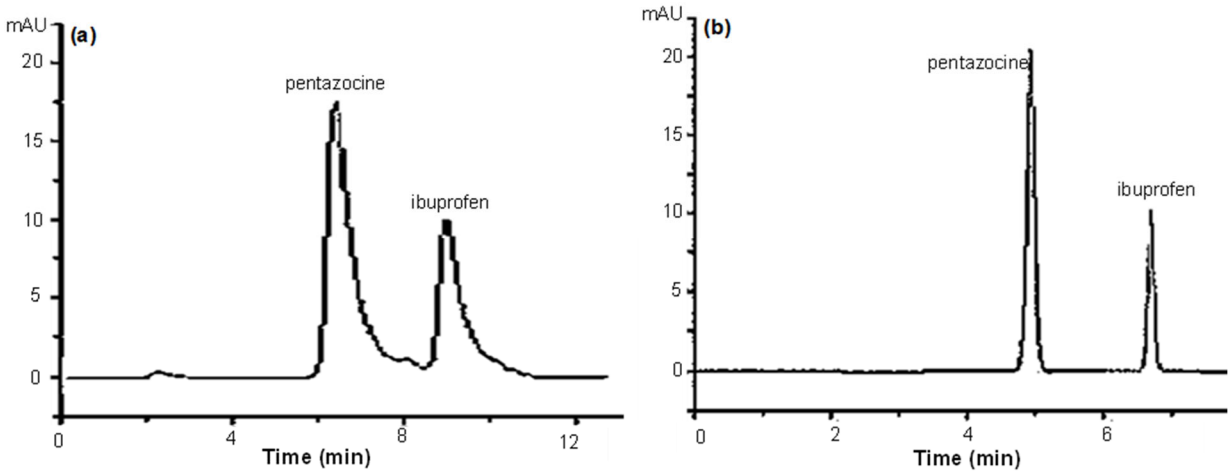

Fig 8. HPLC chromatograms for mixture of pentazocine and ibuprofen with 30 µg/L for each, where (a) according to a reference method HPTLC: acetonitrile and water (35:65, v/v), FR 1.0 mL/min, and (b) according to a 2D-HPLC studied method: acetonitrile, water, and phosphoric acid in a ratio of 45:55:0.1, with an FR of 1.0 mL/min

A mixture containing equal amounts of oxycodone and pentazocine at a concentration of 30 µg/L was prepared. This combination is used for severe pain relief. The simultaneous analysis of the three compounds was conducted according to the previously described method [29]. In short, an RP-18e column was used with an eluent solution consisting of a mixture of acetone and water in a ratio of 60:40, adjusted to pH 3.5 using 0.1% formic acid, at a FR of 1.5 mL/min. The detection wavelength was set at 254 nm, which corresponds to pentazocine, Fig. 9(a).

The results indicate a relatively long retention time with significant peak overlap, which does not allow simultaneous analysis to be performed using this method. When analyzing the above mixture using the studied technique in this research, Fig. 9(b), we found a decrease in the retention times for the two studied compounds and the maintaining peak separation. Additionally, the peaks became more symmetric and narrower, which allows for simultaneous and highly efficient analysis of these three compounds.

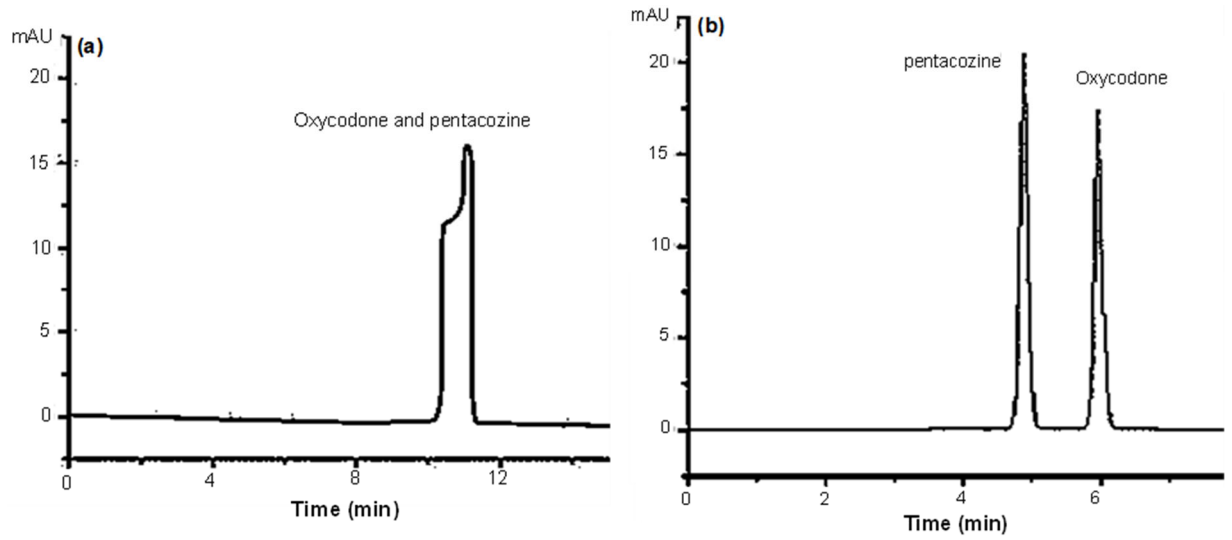

Fig 9. HPLC chromatograms for mixture contain oxycodone and pentazocine with 30 µg/L for each, where (a) according to a reference method HPTLC: acetone, water (60:40), FR of 1.5 mL/min and (b) according to a 2D-HPLC studied method: acetonitrile, water, and phosphoric acid in a ratio of 45:55:0.1, with an FR of 1.0 mL/min

Method Application

The advanced experiment was applied to real pharmaceutical substance samples and produced good results (Table 3). The observations indicated the effective utilization of 2D-HPLC method in pharmaceutical samples without any interference from other drug components (Fig. 10). Pentazocine samples, including ampoules (30 mg/mL), and hydrochloride-acetaminophen tablets (25/650 mg), were analyzed using the developed method at various concentrations. Results, as shown in Table 3, indicated high precision and accuracy, with RSD from 1.05 to 1.40% for within-day and 1.27 to 1.31% for between-day measurements, and recovery percentages ranging from 99.2 to 100.1%. The validity method is confirmed through comparison results by those obtained with the ICH reference method, a standard HPLC technique used for calibrating and determining concentrations in pharmaceuticals containing pentazocine ensuring quality and safety [29].

The statistical analyses by use of t-tests and F-tests were done on pentazocine, Talwin, and pentazocine hydrochloride and acetaminophen tablets to compare the proposed analysis method with the method reference (Table 4). To all samples (n1 = n2 = 5) the degrees of freedom were 8 for t-tests and 4 for F-tests of minimum tabulated values of 2.31 for t-tests and 2.77 for F-tests at a 95% confidence level.

In each sample, the t-test and F-test values were lower than the minimum tabulated values: pentazocine (t-test: 0.37, F-test: 0.71), Talwin (t-test: −0.23, F-test: 0.18), and pentazocine hydrochloride and acetaminophen tablets (t-test: 0.12, F-test: 0.55). This indicates there is no significant difference statistically between the proposed analysis and reference method in all cases, suggesting the compatibility and accuracy of the proposed method in

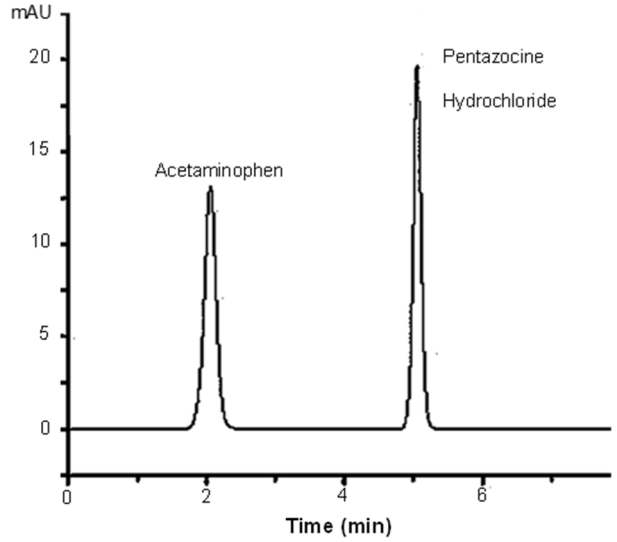

Fig 10. HPLC chromatograms of pentazocine hydrochloride and acetaminophen, The eluting agent was a combination of acetonitrile, water, and phosphoric acid in a ratio of 45:55:0.1, with a FR of 1 mL/min, and a temperature of 25 °C

Table 3. 2D-HPLC method results for pharmaceutical samples containing pentazocine

Samples	Taken µg/L (n = 5)	Found µg/L $\bar{x} \pm S$	R (%)	RSD (%)
Pentazocine (pure)	30	29.94 ± 0.31	99.8	1.05
Pentazocine (ampule) 30 mg/mL	30	29.82 ± 0.42	99.4	1.40
Talwin (ampule) 30 mg/mL	30	29.76 ± 0.38	99.2	1.27
Pentazocine hydrochloride and acetaminophen tablets 25/650 mg	30	30.03 ± 0.40	100.1	1.31

Table 4. Comparing of results among the proposed 2D-HPLC method and the ICH reference method for pharmaceutical samples containing pentazocine

Samples	Suggested method (n = 5)	Reference method (n = 5)	F-test	T-test
	Found µg/L, $\bar{x} \pm S$			
Pentazocine (ampoule) 30 mg/mL	29.82 ± 0.42	29.52 ± 0.62	0.71	0.37
Talwin (ampoule) 30 mg/mL	29.76 ± 0.38	30.02 ± 0.78	0.18	-0.23
Pentazocine hydrochloride and acetaminophen tablets 25/650 mg	30.03 ± 0.40	29.95 ± 0.52	0.55	0.12

measuring active substance concentrations, affirming its quality and alignment with the reference method.

Data Analysis

The 2D-HPLC method, validated per ICH guidelines, exhibited excellent selectivity with pentazocine peaks at 5.0 min, clear of excipient or degradation interference (Fig. 5 (b). It showed strong linearity (R^2 = 0.998) across 10–150 µg/L (Fig. 6), and high sensitivity with LOD and LOQ at 1.58 and 4.79 µg/L, respectively, making it effective for pentazocine quantification. The method displayed high reproducibility with intra-day and inter-day precision, where RSDs ranged from 1.02 to 4.20% and 1.27 to 4.82%, respectively, well within the ICH guideline of 5% (Table 2). Recovery values between 97.0 and 102.5% met the 98–102% acceptance criteria, affirming its accuracy. The developed 2D-HPLC method successfully distinguished pentazocine from co-formulated drugs, outperforming the reference HPTLC method by resolving overlapping peaks of pentazocine, paracetamol, and caffeine (Fig. 7). It also effectively separated ibuprofen and oxycodone mixtures, where reference methods showed overlap (Figs. 8(b) and 9(b)).

The validated method was successfully applied for the quantification of pentazocine in three pharmaceutical formulations – pentazocine ampoule, Talwin ampoule, and pentazocine hydrochloride plus acetaminophen tablets. The recovery values were between 99.2–100.1% with RSD lower than 1.5% (Table 3), demonstrating the accuracy and precision of the method. Statistical comparison using t-test and F-test showed no significant differences between the results from the proposed 2D-HPLC method and the standard ICH HPLC method (Table 4). The t-test values (0.120.37) were lower than the tabulated t-value (2.306), while the F-test values (0.18–0.71) were lower than the tabulated F-value (2.77). This proved that the developed 2D-HPLC method is equivalent in performance to the standard HPLC method for the determination of pentazocine in pharmaceuticals.

The 2D-HPLC method is advantageous due to its ability to combine different separation mechanisms, increasing selectivity and efficiency. The validation of this method ensures its suitability for quality control testing and its transferability across different laboratories [30]. In Table 5, key aspects of various scientific studies are succinctly summarized, highlighting their methodologies, findings, and applications in the fields of pharmaceutical and analytical sciences.

Table 5. Comparative analysis of pharmaceutical studies and techniques

Focus	Method	Key findings	Applications	Ref.
Producing of spherical gold nanoparticles (AuNps).	The chemical method used tranexamic acid as a reducing and capping agent.	Tr-AuNps with a surface plasmon absorption band in 522 nm. Superior selectivity for detecting NA in serum and urine.	Quantification of NA with human blood and urine samples with high recuperation rates.	[11]
Voltammetric behavior of nalbuphine hydrochloride (NP·HCl).	Utilization of cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV).	Distinct voltammetric peaks for NP·HCl in B-R buffer solution using PGE and GCE electrodes. Quantitative determination in pharmaceutical and human biological fluids.	Quantitative determination of NP· HCl in pharmaceutical and human biological fluids.	[12]
Binding assay using mass spectrometry for human µ-opioid receptor.	LC-MS/MS.	Development of a non-radioactive binding assay for opiates/opioids at the human µ-opioid receptor. Identification of femtogram quantities of DAMGO. Consistency with radioactive receptor binding studies. Ki values for 17 opiates/opioids and 6 2-benzylbenzimidazoles.	Ascertainment of Ki values of opiates/opioids, including designer opioids like isotonitazene. Alternative to radioactive binding assays for assessing receptor binding affinities.	[13]

Focus	Method	Key findings	Applications	Ref.
Binding assay using mass spectrometry at the human μ-opioid receptor.	LC-MS/MS	Non-labelled DAMGO used. Minimal non-specific binding. The equilibrium dissociation constant of DAMGO was 0.57 nM. Ki values of 17 opiates/opioids and six 2-benzylbenzimidazoles ranged from 0.654 to 72.90 nM.	Ascertainment of Ki values of opiates/opioids, including designer opioids like isotonitazene. Alternative to radioactive binding assays for assessing receptor binding affinities.	[14]
Spectrophotometric techniques for quantification of four analgesic medications (NALB, NALT, MORF, TRAM) in pharmaceutical formulations.	Spectrophotometric methods involving *N*-bromosuccinimide with methyl orange (method A) or orange G (method B).	Beer's law limit, molar absorptivity, and Sandell's sensitivity computed. Methods A and B effectively quantify analgesic medications in pharmaceutical formulations. The presence of common additives did not interfere.	Quantification of NALB, NALT, MORF, and TRAM in pharmaceutical formulations.	[15]
Gas-liquid chromatography (GLC)technique for identification of pentazocine, pheniramine, and cotinine in urine.	Capillary column GLC.	Linearity from 50 to 1000 ng/mL with a correlation coefficient exceeding 0.999. LOQ of 50 ng/mL for each medication. Efficacious in examining specimens from individuals indicating drug misuse.	Identification and surveillance of pentazocine, pheniramine, and cotinine use in clinical settings for addiction treatment and monitoring.	[16]
Examination of the misuse of pentazocine and antihistamines among opioid-addicted individuals; use of capillary column GLC for drug identification in urine.	Capillary column GLC technique for simultaneous identification of pentazocine, pheniramine, and cotinine in urine.	Urinalysis plays a critical role in the treatment of drug use, particularly for pentazocine and antihistamines among opioid addicts.	Identification and monitoring of drug misuse, particularly in opioid-addicted individuals.	[17]
Simultaneous analysis of 30 types of analgesics and adjuvant analgesics in serum using LC/TOF-MS.	Solid-phase dispersive extraction (SPDE) for serum pretreatment and LC/TOF-MS for analysis.	High recovery rates (49–112%) for analgesics in serum samples with and without deproteinization. Matrix effect consistent regardless of deproteinization. Minimum detectable amounts between 0.25 and 10 ng/mL. Strong correlation (over 0.998) between measured values and concentrations.	Detection of pain relievers and adjuvant pain relievers in serum, useful in forensic science and emergency medicine.	[18]
Development of PET imaging agents (−)- and (+)-[11C] OMDV for detecting synaptic alterations in Alzheimer's disease and measuring binding affinity to the vesicular acetylcholine transporter (VAChT).	HPLC separation into (−)- and (+)-optical isomers of OMDV and OTDV; *in vitro* and *in vivo* assessments of binding affinity and brain uptake; PET-CT imaging for *in vivo* studies.	(−)-OMDV showed an eightfold higher binding affinity to VAChT than (+)-OMDV. Both enantiomers penetrated the blood-brain barrier, but (+)-OMDV cleared faster from the brain. Vesamicol reduced the accumulation of (−)-[11C] OMDV in the cortex, while (+)-pentazocine and (+)-3-PPP did not significantly affect uptake.	PET imaging for examining presynaptic cholinergic neurons in the brain, particularly for Alzheimer's disease research.	[19]
Rapid estimation of oxymetazoline and isoxsuprine hydrochloride in pharmaceutical compositions using HPLC.	HPLC with an Azorbax-C8 column and a mobile phase comprising methanol, acetonitrile, and buffer. Detection using a photodiode array detector.	Retention times of 3.6 ± 0.4 min for isoxsuprine HCl, linear range of 1–250 μg/mL, and detection limits of 0.5 μg/mL for both drugs. Accurate measurement of ISX and OXY levels.	Estimation of oxymetazoline and isoxsuprine hydrochloride in pharmaceutical compositions for quality control.	[20]

Focus	Method	Key findings	Applications	Ref.
Development of a 2D-HPLC method for accurate pentazocine quantification in pharmaceuticals.	Utilizes two C18 columns and optimized mobile phase for enhanced resolution and selectivity.	High accuracy, precision, and selectivity in pentazocine detection; outperforms conventional HPLC methods.	Useful for quality control in pharmaceuticals, ensuring correct dosage and patient safety.	Our study

■ CONCLUSION

The conducted study successfully demonstrated the efficacy and reliability of the developed 2D-HPLC method for the quantitative analysis of pentazocine in pharmaceutical compositions. The method exhibited higher accuracy, precision, and selectivity, meeting the stringent acceptance criteria set forth by the ICH guidelines. Notably, the approach showed remarkable linearity of a correlation coefficient exceeding 0.999 and a satisfactory LOQ at 50 ng/mL for each analyzed medication. Additionally, the application in clinical settings of the surveillance and treatment of individuals with drug misuse indications is efficacious and cost-effective. The method outperformed traditional normal phase HPLC techniques demonstrating its suitability for routine quality control analysis for the pharmaceutical industry. Based on the findings, it is recommended the validated 2D-HPLC method be adopted as a standard procedure for the routine quality control of pentazocine in pharmaceutical industries due to its superior sensitivity and specificity. Further research and method development encouraged the extension of this approach in other pharmaceutical compounds, enhancing the scope of this technique with drug analysis. Clinical settings combine this method for the accurate and efficient monitoring of medication levels in patients, particularly in the context of addiction treatment and management. This method having met all validation key characteristics, stands as a strong and dependable tool in the pharmaceutical industry and healthcare providers ensuring the precise dosage and safety of medications to patients.

■ CONFLICT OF INTEREST

There are no conflicts of interest to declare in relation to this study. All aspects of the research were conducted impartially, without any influence from external parties or personal or financial interests.

■ AUTHOR CONTRIBUTIONS

As the sole author, I was responsible for all aspects of this research, including conceptualization, methodology, data collection and analysis, writing, and revising the manuscript, ensuring its overall integrity and coherence.

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