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Stability Evaluation of Intravenous Admixtures of Tramadol Hydrochloride and Acetaminophen at Different Storage Conditions

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ABSTRACT

Objective: This study is particularly performed to analyze the stability, whether an admixture containing ready-to-use solution of Tramadol HCl and Acetaminophen will be stable and compatible for a specified time. As intravenous admixture of Tramadol hydrochloride and Acetaminophen is normally used in hospitals for managing postoperative pains. Therefore, compatibility and stability of these admixtures need to be evaluated experimentally.

Methodology: Five different intravenous fluids were selected for the preparation of admixtures of these two drugs and studied at 5˚C and room temperature (20 – 25˚C) for one week time. Admixtures were evaluated visually for color change, turbidity, precipitation and fluctuation of pH at day 0,1,3,5 and 7. By employing UV/Visible spectrophotometer, percentage purity of both drugs was determined in admixture by applying Simultaneous Equation.

Results: All admixtures were found to retain their physical and chemical characteristics during the definite period of study. Furthermore, strengths of both drugs were persisted more than 90%. Therefore, admixtures of Tramadol HCl and Acetaminophen in various intravenous fluids were found to be stable and compatible when stored for 07 days at 25˚C and at 5˚C.

Conclusion: From the significant results of our work, it is suggested that ready to use of the admixture of Tramadol and Acetaminophen could be prepared and stored in advance for efficient and effective management of pain in busiest pharmacies and hospitals.

Keywords: Tramadol HCl, Acetaminophen, admixtures, simultaneous equation, analgesia.

INTRODUCTION

Tramadol is opioid analgesics which is synthetically prepared and used to manage the severe and moderate pain. Because of bit difference of its mechanism of action and adverse effects to opioid analgesics, Tramadol is named atypical opioid because FDA graded it opiate analgesics in 1995. It was first time synthesized in 1962 and marketed in 1977 by the trade name of TRAMAL. Tramadol chemically is (±) cis [(Dimethylamino) methyl]-1-3-methoxyphenyl) cyclohexanol HCl which is analogue

of codeine. It is an alkaloidal compound and on chemical reaction produces two isomers; (+) and (-) racemate in equal ratio (50 : 50), however, (+) enantiomer is four times more therapeutically effective than (-) one[1].The separation of its enantiomers was done using capillary electrophoreses in sulfated cyclodextrin[2]. The exact mechanism of action is not properly acknowledged, though, it was recognized through animal studies that drug and its metabolites bind to μ-receptor as well as reuptake inhibiting effect of serotonin and nor-epinephrine[3].

The comparative analgesic potential of Tramadol is associated to their relative plasma concentration. In therapeutic dose, it does not affect the ventricular function and histamine release like morphine, instead orthostatic hypotension has been noticed. After oral administration, its total bioavailability was found around 72-75 % and peak serum level was attained within 2-3 hrs [4].The bioavailability of Tramadol varies as per route of administration and found higher plasma concentration through rectal route because of least first pass metabolism. As this drug cross blood-brain barrier (BBB)and spread through breast milk therefore, it is contraindicated in pregnancy and breast feeding women. The metabolism of Tramadol takes place mostly in the liver and 05 different metabolites like M1, M2, M3, M4 and M5 are formed. As concern the drug-interactions, in combination with CYP_2D_6 inhibitors, Tramadol's metabolism was decreased and resulting parental drug raised and metabolites amount lowered in the plasma. Food has no any significant incompatibility with Tramadol, thus can be used safely in both before and after meal situations[5].Tramadol and its metabolites are mainly excreted through kidney and to some extend through feces too. Its serum ½ life is 6.3 hrs which may rise (7.9 – 8.8 hrs) with sustained release dosage form.

The best choice of Tramadol is for the management of postoperative analgesia with minor side effects that can be easily coped. Neuropathic pain can also be well managed with Tramadol in different therapeutic ailments such as; nutritional deficiency, malignancy, diabetes and HIV. It can be employed safely for the treatment of chronic cases (osteoarthritis) for long time, because of its least gastric irritations. Tramadol is more beneficial to detoxified the withdrawal symptoms associated to narcotics (heroin) [6]. The therapeutic oral dose for adults is 100 mg/day in 04 divided doses and maximum up-to 400 mg/day. For the treatment of post-operative pain, parentally administered 100 mg as bolus dose followed by 50 mg after every ½ an hr up-to 250 mg maximum. Pediatric oral and parentral doses of Tramadol are usually 2-3 $mg.kg^{-1}$. Various dosage form of Tramadol is available like; tablets (sustained and immediate release), capsules, suppositories, injections and oral drops. In addition, transdermal patches, creams, gels (topical), solutions and rectal foams [7].Tramadol is μ-opioid agonist which is connected to the existence of withdrawal symptoms (insomnia, hallucinations, sweating) and physical dependence on sudden cessation of drug. However, these syndromes can be overcome when taper the dose[8].

Acetaminophen is centrally acting analgesic and antipyretic moiety (synthetic) which belongs to non-opioid class. In late 19^{th} century its analgesic characteristics were identified and clinically employed by Von Mering [9]. In USA, Acetaminophen was first time marketed in 1953 and included in British Pharmacopoeia in 1963 then rapidly boomed as an over the counter analgesic worldwide. The physical appearance of Acetaminophen is white-crystalline powder and soluble in water (12.78 mg/ml) at 20˚C. It is acetanilide's derivative and showed 6 pH in aqueous solution[10]. Chemically it is called Paracetamol and acetyl para aminophenol. Acetaminophen showed its action by minor inhibition of COX-1 and COX-2. The inhibition of COX-3 in CNS was also recognized. Its antipyretic effect was due to its thermoregulatory response on brain cells, which inhibited the synthesis and release of prostaglandins. Acetaminophen recommended for the treatment of myalgia, headache and postpartum pain as well as drug of choice for patients with gastritis and allergic to aspirin. In addition, chronic-arthritis was managed adequately in combination therapy with anti-inflammatory drugs [11].

The acclaimed adult oral dose of Acetaminophen was 650-1000 mg 4-6 hourly but not more than 4000 mg/day. Parenteral dose used 12.5 mg/kg and not beyond to 3.7 g/day. The daily recommended oral dose for children (less than 12-year) was 10-15 mg/kg 4 – 6 times daily and not exceeded to 75 mg/kg per day [12]. The offered dosage forms of Acetaminophen included; tablet, syrup, oral drops, suspension, injectables and suppositories. Commercially combination product of Acetaminophen with doxylamine, codeine, dihydrocodeine, Tramadol and caffeine also found. The absorption of

Acetaminophen occurred in gastro intestinal track (GIT) through passive diffusion and after 6-hr of oral administration, the peak plasma level was attained (1-4 µg/ml). The presence of food, affected the gastric absorption of Acetaminophen, therefore parenteral route showed extensive therapeutically than orally administered drug [13]. Drug passed through placental barrier and within half an hour it reached to fetus where successfully metabolized by glucuronidation. As little amount (0.1 – 0.85 %) of drug excreted through milk so, no any serious interruption have seen with maternal dose of Acetaminophen. Metabolism of Acetaminophen takes place in 03 modes of way such as; N-hydroxylation, sulfonation and glucuronidation and approximately 90 % metabolized. Around 5 % oxidized to benzoquinone imine which allied to the liver toxicity. Only 5% of the drug is excreted as unaffected through kidneys [14].

The individual and combined therapeutic response of Tramadol and Acetaminophen were determined and found combination therapy was quite aggressive in post-operative pain management. Consequentially, it was perceived the better patient compliance and drop of hospitalization time in combination therapy [15]. On comparison the doses of Tramadol and collective preparations, low dose of combined Tramadol-Acetaminophen was found equally effective to control the postoperative pain (spinal surgery) than Tramadol only. The cost effectiveness was the main issue in combined form [16]. The better and safely control of analgesia in post cesarean patients was observed in conjunction remedy than separable Tramadol and Acetaminophen. In addition least side effects were noticed with combination form [17].The efficacy of combined Tramadol-Acetaminophen and other analgesics was determined to manage the back pain of cancerous patients, the multi-drug therapy was found more superior to other solitary therapeutic compounds [18]. When used combination of analgesics (i.v.) with diverse modes of mechanism to control moderate to severe pains are termed as multimodal analgesia (balanced-analgesia). Accordingly, there was higher analgesic effect and minor adverse effects have seen by employing this strategy of treatments. Hence, combination therapy of intravenous admixture was preferred to manage severe and moderate aches [19]. Multimodal analgesic procedure in recent years exhibited the most significant, comparatively safe, compliance benefits and low dose of drugs comparative to individual composites [20]. The most appropriate way of applying multimodal analgesia is, in which more than one drug administered intravenously in the same container. However, it must be ensured that contents of admixtures must be stable and compatible as well as retained their therapeutic potential during the preparation and storage. The stability issue is mainly associated to the temperature and light which consequently fluctuates the pH and solubility of drugs and their additives. Two types of instabilities have found in intravenous admixtures such as; chemical and physical instabilities. The mixture of incompatible drugs caused the chemical instabilities which may be due to the oxidation, reduction and hydrolysis of drug's components. If one or more component decomposed (>10 %), the admixture was supposed to be chemically unstable. This type of instabilities was determined through analytical techniques. Physical instabilities were identified in the form of color-change, crystallization, turbidity and precipitation, which may not have any serious destructive effects[21].

The objective of the study was to evaluate the stability of Tramadol in 5 % Dextrose solution kept for the period of fortnight. Hospitalized patients were generally treated with Tramadol HCl and its admixtures with other analgesics. The concentration of Tramadol was determined and observed within the normal range. However pH of the solution was minutely decreased after the specific storage admixture. Consequently, the Tramadol in 5 % dextrose IV infusion could be prepared in advance for the utilization in hospitals [22].The stability of Tramadol admixture with tromethamine, ketorolac, metoclopramide and magnesium sulphate was assessed using HPLC technique after 05 days of preparation and stored at 5˚C and 25˚C temperatures. Drugs were found to retain their potency (98 %) and it was concluded that such combination must be considered for the preparation in advance [23]. This study was focused on the stability study of ketoprofen and Tramadol admixture stored at 20˚C – 25˚C without adjustment of pH and evaluated with HPLC. Results showed that admixture of these 02 drugs may well be prepared and used after 07-day of shelf life safely [24].

As, this combined therapy (Tramadol and Acetaminophen) was preferably recommended to treat moderate to severe pain. The aim of this study was to analyze the stability of intravenous-admixture

of Tramadol (a centrally acting) and Acetaminophen (acting peripherally) and synergistic effect with low dose of Tramadol HCl which successively diminished its side effect. Degradation of products with least effect were noticed [25]. This study was focused on the stability study of ketoprofen and Tramadol admixture without adjustment of pH. Results showed that admixture may be prepared and used safely after 07-day of shelf life. Therefore, this combination therapy exhibited significant stability profile and can be prepared in bulk quantity (in advance) particularly in large hospitals.

MATERIAL AND METHODS

Preparation of intravenous admixtures from Intravenous infusions

Intravenous infusions are used for the various purposes such as; replacement of fluids and electrolytes, as diluents for the intravenous admixtures' preparation. The stability of Tramadol/Acetaminophen admixture was studied in 05 different isotonic solutions [26].

Techniques employed for the evaluation of Stability

Visual Method

The physical instability of admixture is generally measured by the change of its appearance, which usually evaluated by the visual-methods like; precipitation, color and turbidity.

Determination of pH

The acid base characteristics of drug products are associated to the change in pH and stability of solution. The degree of hydrolysis catalyzed by H and OH ions which is also linked with pH change.

Spectrophotometric Analysis

Double beam UV/Visible spectrophotometer (Shimadzu UV-2550) was used to determine the absorbance, which provides high energy throughput (200-600nm) and dynamic ranges.

Calibration Standard of Tramadol HCl

Dissolved 100mg of Tramadol in 1000ml of N/10 NaOH solution (Stock-solution). Working solution of various strengths was prepared by dilution of stock solution with NaOH (N/10), such as; 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml.

Standard solution preparation

Took accurately weighed (100mg) Tramadol HCl in 1000ml volumetric flask. Add 100ml (0.1 N) NaOH solution, then made volume up-to 1000ml with the same solvent and filtered. The resulting concentration of filtrate was 0.1mg/ml.

Sample solution preparation

In 100ml measuring flask add 2ml of Tramadol injection and 50 ml 0.1 N NaOH, mixed thoroughly in ultrasonic bath. Made the volume up-to the mark with 0.1N NaOH and filtered. Consequently, 0.1mg/ml concentration was attained.

Analytical Procedure

The Absorbance of standard and sample solutions was measured separately using Spectrophotometer at 271nm. NaOH (0.1 N) solution was used as blank.

Calculations

Using the following formula, percentage purity of Tramadol HCl was determined.

Absorbance of sample solution

$$\text{Percentage Purity of Tramadol HCl} = \frac{\text{Absorbance of sample solution}}{\text{Absorbance of standard solution}} \times 100$$

Calibration of Standards of Acetaminophen

Dissolved 300mg of Acetaminophen pure analytical grade powder in 100ml methanol called stock solution. Then different concentrations were prepared from this stock solution using deionized water, like; 0.1, 0.2,0.3, 0.4, 0.5 and 0.6 mg ml^{-1}.

Preparation of Standard Solution of Acetaminophen

Weighed 300mg of Acetaminophen reference and transferred to 100ml volumetric-flask. Added 50ml methanol into the flask and dissolved the powder, then made volume up-to the mark. Took 01ml solution in another 100ml measuring-flask, added 0.5ml of 10.3g/L of HCl solution and finally made up-to 100ml with methanol. The final strength of solution was 0.03mg ml^{-1}. Other dilutions of standards were also prepared.

Preparation of Sample Solution

Mixed 2ml of Acetaminophen solution (injection) with 50ml of methanol in 100ml measuring-flask and volume was made up-to the mark with methanol. Stock solution was pipetted out (01ml) in 100ml volumetric flask, added 0.5ml 10.3g/L sol of HCl and

sufficient quantity of methanol up-to the mark (0.03mg ml^{-1}).

Analytical Protocol and Calculations

Measured absorbance of sample and reference at 257 nm using UV-Spectrometer in triplicates. The percentage purity of Acetaminophen was calculated using formula.

$$\text{Percentage Purity of Acetaminophen} = \frac{\text{Absorbance of sample solution}}{\text{Absorbance of standard solution}} \times 100$$

Simultaneous Determination of Tramadol HCl and Acetaminophen

Preparation of Standard Solution

300 mg of Acetaminophen standard was taken in measuring flask (100 ml) and added 50 ml of NaOH (0.1 N) to it. As compound was properly dissolved, took 01 ml of this sol in to another 100 ml volumetric flask and 100 mg of Tramadol HCl standard was also added. Then added 0.1 N solution of sodium hydroxide and dissolved Tramadol HCl and lastly made the volume up-to 100 ml with additional NaOH (0.1 N)solution. Solution was filtered as in previous protocol. Consequently, 0.1 mg/ml and 0.03 mg/ml concentration of Tramadol and Acetaminophen were achieved respectively.

Calculations

The UV absorbance of admixture having Tramadol and Acetaminophen was perceived at 257 nm for Acetaminophen and 271 for Tramadol HCl with NaOH (0.1 N) as blank. Concentration of both drugs was calculated using the following simultaneous equation.

$$\text{Concentration of Tramadol HCl} = \frac{A_1 \times \text{intercept of } A_1 - A_2 \times \alpha_2}{(\text{Intercept of T} \times \text{Intercept of A}) - (\alpha_2 \times \alpha_1)} \times 100$$

$$\text{Concentration of Acetaminophen} = \frac{A_2 \times \text{intercept of T} - A_1 \times \alpha_1}{(\text{Intercept of T} \times \text{Intercept of A}) - (\alpha_2 \times \alpha_1)} \times 100$$

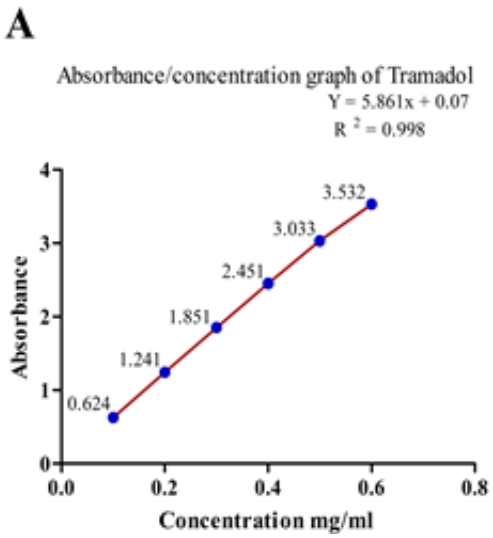
Where

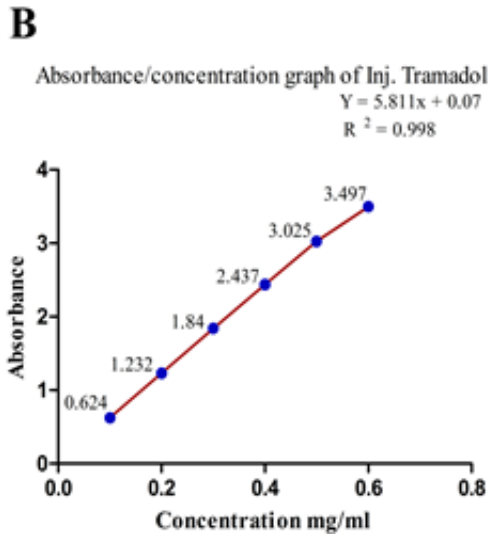
T: Tramadol HCl
A: Acetaminophen
A_1: Absorbance at λ_1
α_1: Absorptivity of Acetaminophen
A_2: Absorbance at λ_2
α_2: Absorptivity of Tramadol HCl

RESULTS

Determination of Standard Curve of TramadolHCl (Reference),Inj. of Tramadol and UV Spectrum with statistical comparison

Took the absorbance of all standard solutions of Tramadol reference and injection and finally plotted curve against respective concentrations (0.1 – 0.6 mg/ml). The calibration curve of standard Tramadol and its injection was illustrated in Figures **1A** and **1B**, which showed the progressive rise of absorbance with increase the Tramadol concentrations according to the linear regression. The spectrum of Tramadol which showed λmax and comparative data presented in Figure **2A** Table **1**.





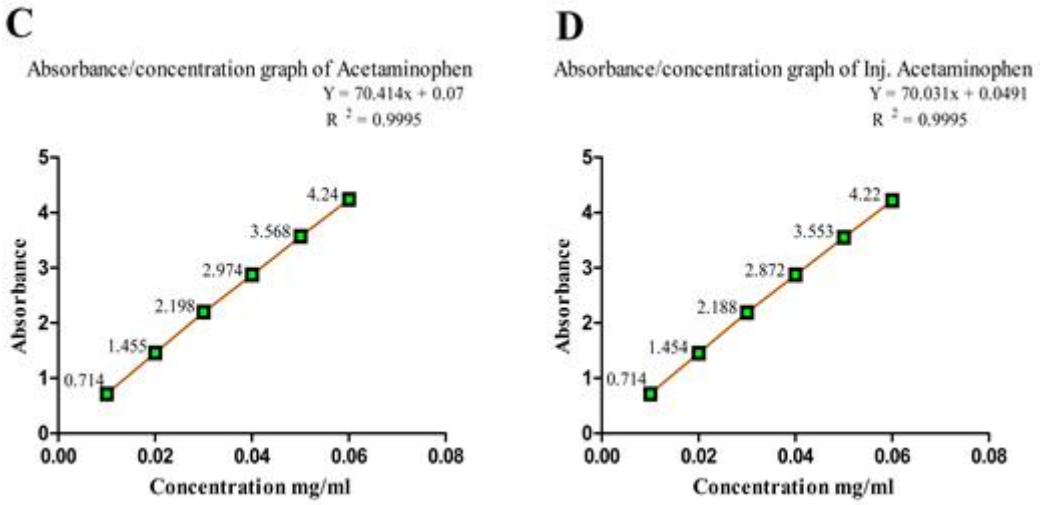


Figure 1. Standard Curve of Tramadol Standard **(A)** (Concentration vs Absorbance), standard curve of Tramadol inj**(B)**, absorbance of Acetaminophen Standard at different concentrations **(C)**, absorbance of Acetaminophen Inj at different concentrations **(D)**

Table 1. Statistical parameters of Standard and Injection of Tramadol.

Statistical Parameters	Tramadol Standard	Tramadol Injection
Slope	5.8617	5.8117
Y Intercept	0.047	0.043
Average Absorbance	2.122	2.1091
RSQ	0.998	0.998
Standard deviation	1.0972	1.0881

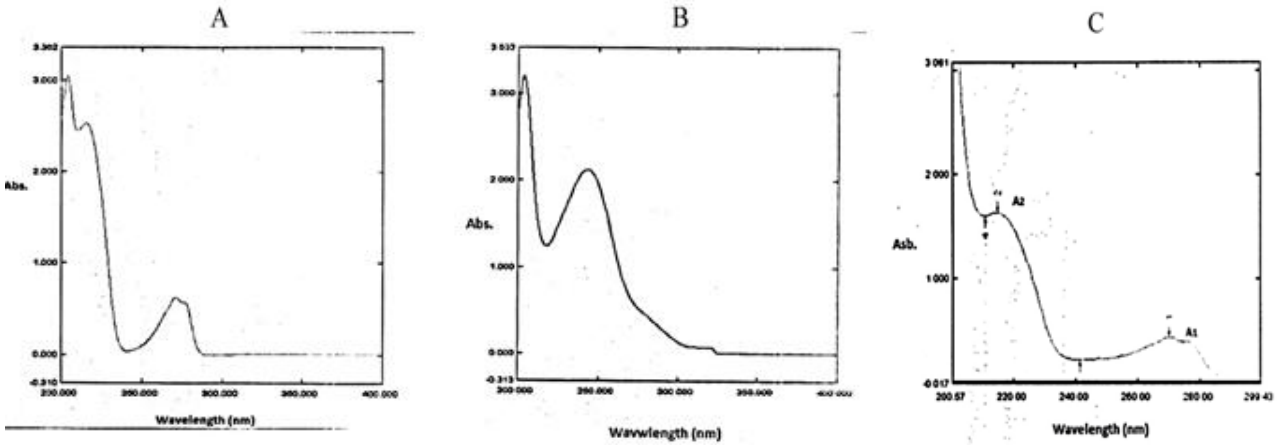


Figure 2. UV Spectrum of Tramadol HCl at λ max: 270 nm **(A)**, UV Spectrum of Acetaminophen Standard **(B)**, UV-Spectrum of Tramadol HCl and Acetaminophen mixture at λ1 210 & λ2 at 271 **(C)**

Determination of Percentage Purity of Injection Tramadol

The absorbance of 0.1mg/mL concentration of Tramadol Standard and Injection was determined (0.624) as given in Tables 1 and 2. The percentage purity of Tramadol injection was evaluated as.

$$\text{Percentage Purity of Tramadol HCl} = \frac{0.624}{0.624} \times 100 = 100\ \%$$

The % age purity of Tramadol Inj. is within limits (99 – 100%)

Table 2. Statistical Considerations of Acetaminophen Standard and Acetaminophen inj

Statistical Parameters	Acetaminophen Standard	Acetaminophen Injection
Slope	0.7035	0.7042
Y Intercept	0.0700	0.0701
Average Absorbance	2.509	2.508
RSQ	0.9995	0.9995
Standard deviation	1.3163	1.3176

Table 3. Statistical parameters of Tramadol HCl and Acetaminophen Standard

Statistical Parameters	Tramadol Standard	Acetaminophen Standard
Slope	5.8617	0.7035
Y Intercept	0.047	0.07
Absorptivity	1859.57	7215.37
Molar Concentration	0.00033	0.00019
R^2	0.999	0.998
Slope	5.8617	0.07

Table 4. Percentage Recovery of Tramadol HCl and Acetaminophen Standards from Binary mixture

Drug Compound	Amount added	Amount recovered	Percentage Recovery
Tramadol HCl	0.1 mg/ml	0.1002	0.1002/0.1 = 100 %
Acetaminophen	0.03 mg/ml	0.030	0.030/0.031 = 100 %

Evaluation of Acetaminophen (Reference), injection of Acetaminophen, UV Band and study of statistical data.

The absorbance against corresponding concentration (0.01 – 0.06 mg/mL) was given in Figures **1C**, **1D**, **2B** and Table **2**.

Percentage purity of Acetaminophen Injection

The absorbance of Acetaminophen standard and injections were found 0.715 and 0.714 respectively. The percentage purity was calculated by the formula.

$$\text{Percentage Purity of Acetaminophen} = \frac{0.714}{0.715} \times 100 = 100\ \%$$

Acetaminophen concentration of injection was found to be within limits (99 – 100 %)

InstantaneousDetermination of Tramadol and Acetaminophen

Tramadol and Acetaminophen Binary Solution Preparation

Scanned the binary solution at wavelength range of 400 – 200nm and found maximum absorptions (λmax) at 271 and 210nm as shown in Figure **2C**. The comparative statistical parameters were analyzed in Table **3**. The percentage recovery of drugs was determined and illustrated in Table **4**.

Percentage Recovery of Tramadol and Acetaminophen Standard from Binary Mixture was determined as;

$$\text{Concentration of Acetaminophen} = \frac{A_2 \times \text{intercept of T} - A_1 \times \alpha_1}{(\text{Intercept of T} \times \text{Intercept of A}) - (\alpha_2 \times \alpha_1)} \times 100$$

$$= \frac{0.624 \times 0.047 - 1.432 \times 7215.370}{(0.07 \times 0.047) - (1859.57 \times 7215.37)} \times 100$$

= 0.0301 mg/ml

$$\text{Concentration of Tramadol HCl} = \frac{A_1 \times \text{intercept of T} - A_2 \times \alpha_2}{(\text{Intercept of T} \times \text{Intercept of A}) - (\alpha_2 \times \alpha_1)} \times 100$$

$$= \frac{1.432 \times 0.07 - 0.624 \times 1859.57}{(0.07 \times 0.047) - (1859.57 \times 7215.37)} \times 100$$

= 0.1002 mg/ml

Stability Study of Tramadol HCl and Acetaminophen Intravenous Admixture

Visual Inspection

Visual parameters are usually employed regarding evaluation of physical instability of drug admixtures. Two lots of admixtures were prepared with 05 different solutions and examinations of physical

changes were made on 0, 1st, 3rd, 5th and 7th day. The evaluating parameters were turbidity, precipitation, change of color and crystallization that were perceived against white back-ground.

Visual Inspection of the Admixture Stored at 20˚C – 25˚C

There was no any physical variation like turbidity and precipitation originated in the admixture hold at room temperature. However, ringer lactate containing admixture showed minor color change on 5th to 7th days of storage. In addition, admixture with DW 10 % also revealed same effect on day 7.

Visual Inspection of Admixture Stored at Cool Temperature

The admixture was found to be stable and no any physical changes detected during our work in the admixture that was kept at 2˚C – 8˚C.

pH Determination

According the results illustrated in the graph showed the little fluctuation of pH but the fluid admixtures of Ringer lactate and Dextrose water 10 % stored at room temperature presented bit higher pH at 4th day in RL and 3rd day in D10 % (Figure**3A)**. When pH was observed in the admixture stored at cool temperature and noticed that NS and D10 % fluids had higher fluctuations than other fluids (Figure **3B**).

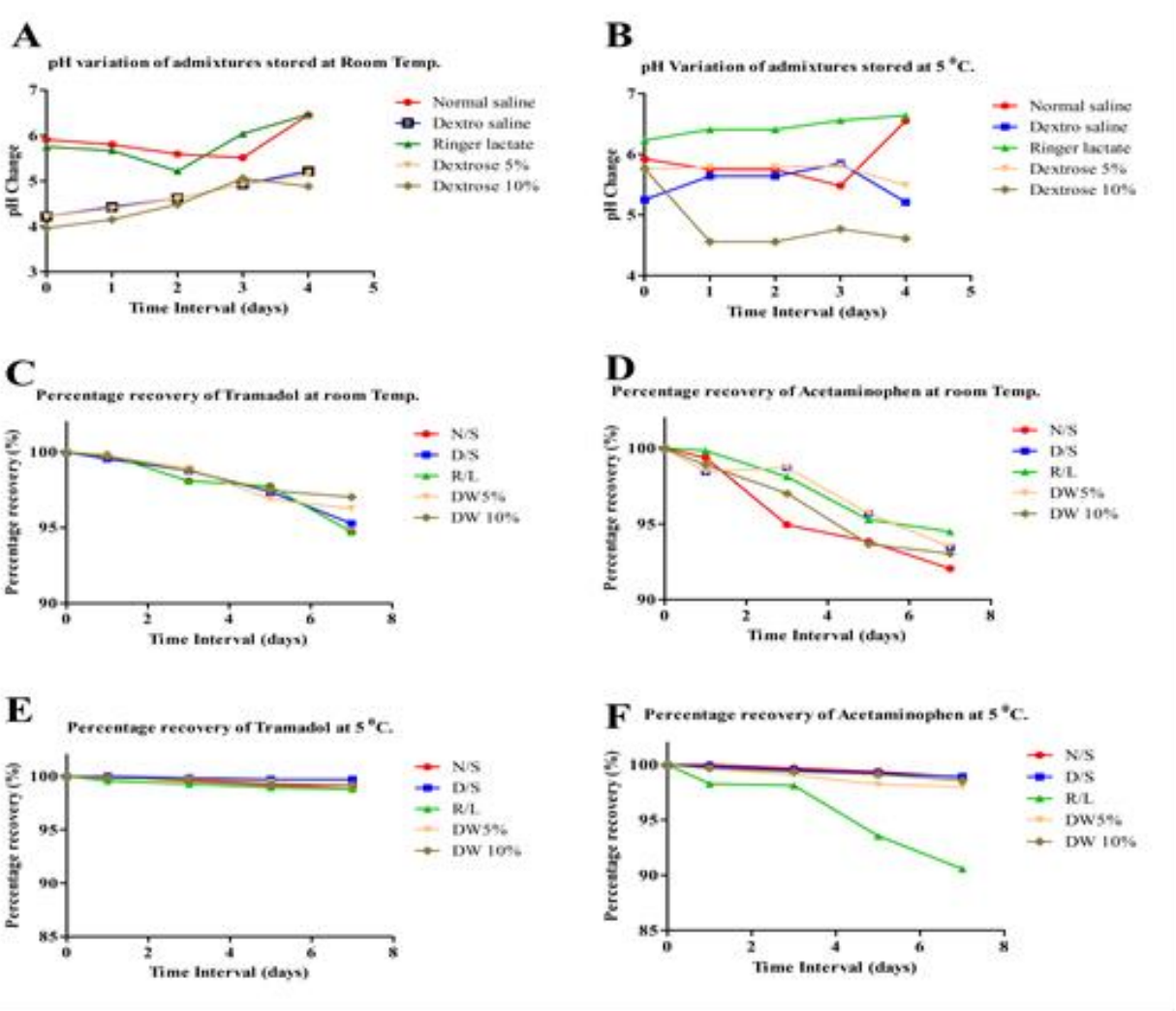


Figure 3.pH at various time intervals of admixture stored at room temperature**(A)**, pH at various time intervals of storage of admixture at 5˚C**(B)**, decrease in Percentage Recovery of Tramadol at Room Temperature**(C)**, decrease in Percentage Recovery of Acetaminophen at Room Temperature **(D)**, reduction in % age recovery of Tramadol at 5˚C with storage **(E)**, reduction in % age recovery of Acetaminophen at 5˚C**(F)**

as

UV/Visible Spectrophotometric Analysis of Intravenous Admixture

Percentage recovery of Tramadol and Acetaminophen admixture at room temperature

The minimum decrease of percentage contents of Tramadol was observed in DW10 % (99.98 to 97.03) during the whole week of experimental time at room temperature. The second least fall was seen in DW5 % fluid (99.98 to 96.26), however, minimum recovery of Tramadol was noticed with R/L (94.72) during the course of time. These results exhibited that % contents of active drug in all five batches was within official limits and significant (Figure**3C**). As concerned the percentage purity of Acetaminophen in the admixture at the day 7 was found maximum lowered in N/S (100 to 94.04) and 2nd decreased amount was noted in DW10 % (93.04), while other fluids showed the mixed but reliable and significant response, according to the FDA profile (> 90 %) as given in Figure **3D**.

Percentage recovery of Tramadol and Acetaminophen admixture stored at 5˚C

The percentage content of Tramadol in different intravenous fluids was evaluated at various time frames from day 1 to day 7, stored at cool temperature (5˚C). Almost all fluids showed very minute decrease in % age concentration such as; 0.28 %and 1.19 % from Dextro saline and Ringer lactate fluids respectively. Ours study showed the recovery of Tramadol in admixtures 2˚C – 8˚C was significantly standard (> 98.8 %) as per FDA guideline that required more than 90 % as indicated in Figure**3E**. The concentration of Acetaminophen under same condition of temperature was found more adequate and above 99.5 % in all experimental admixtures except Ringer lactate which showed 90.57 % as presented in the Figure **3F**.

Analytical Methods and Validation

Specificity and Selectivity

Solutions of standard Tramadol HCl were scanned at 400 – 200 nm. The absorption spectrum of standards and injections were matched and found there was no any significant difference between the average absorbance of reference drugs and formulations. Consequently, planned analytical mode is considered specific and selective.

Linearity

Six series of Tramadol HCl solutions (0.1 – 0.6 mg/ml)were prepared from the stock solutions and investigated. The resulting data was analyzed by least square regression. The linearity range for the above series of solutions was found R^2 = 0.9990. The fit of the regression equations quality was sustained by the high regression-coefficient values.

Accuracy

The reliability and validity of the recommended methods was evaluated by the recovery studies in 0.1 N NaOH, the mean percentage recovery was found to be 100 %. According to the results, any minor change in strength of drug in admixture could be precisely calculated by the projected analytical techniques.

Precision

Using different concentrations of drugs from their stock solutions to performed repeatability. The intermediate precision of analytical methods was determined on the basis of intra-day, inter-day and inter-instrumental variations. The final results presented the SD less than 1 and showed good repeatability.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

By using calibration standards, the LOD and LOQ of Tramadol HCl (Proposed method)were determined as 3.3 σ/S and 10 σ /S respectively. Where, σ is the concentration and S the slope of calibration. Beer's law was followed in the concentration from 0.1 to 0.6 mg/ml (R^2 = 0.9990) in sodium hydroxide (medium). Acetaminophen's method was also validated by applying the same parameters. Considering statistical data (Table **6**), the simultaneous determination methods of Tramadol and Acetaminophen were found to be accurate, specific precise, reproducible and repeatable.

DISCUSSION

The combination of analgesics like Tramadol and Acetaminophen admixture is commonly used in many hospitals to manage the post-operative pain. The basic necessity for the preparation of intravenous admixture is that it should be stable and drugs components must be compatible over the time of administration. There was no any substantial data was existed regarding the stability of this admixture. In order to evaluate the stability profile and shelf life of

intravenous-admixture, we executed this study. UV/Visible spectroscopic analysis of five series 0.1 – 0.6 and 0.01 – 0.06 mg/ml of Tramadol and Acetaminophen were performed respectively. Data was presented through regression line equation using GraphPad Prism-5. For the preparation of admixture of Tramadol and Acetaminophen we used 05 different intravenous fluids such as; Dextrose water 5% (D5), Normal saline (NS), Dextrose Water 10% (D10), Dextrose saline (5,0.9) (DNS) and Ringer lactate (RL).

Evaluation of stability was performed on admixtures through pH-measurement at room temp, physical monitoring and spectroscopic method on 0, 1st, 3rd, 5th and 7th day. There was no any sign of turbidity/precipitation was seen. However, admixtures with DW10% and RL showed the slight color change (yellowish-brown) on 5th day. The pH of all admixtures was found bit fluctuation given in Figure 3A. Vials that hold DW10% and RL also showed higher pH change, therefore there might be some relations between color change and pH growth. UV/Visible spectrophotometer was used to analyze the quantity in admixtures. According to the Figure 3C and average percentage recovery of Tramadol in admixture stored at room temperature was 98.0 – 98.6 %, which was significant in official limits (> 90 %) as per FDA guidelines. The mean % age recovery of Acetaminophen was 96.0 to 96.5 %, which also come in official limits (> 90 %) Figure 3D.

Five intravenous admixtures of Tramadol and Acetaminophen were prepared with five IV. fluids and stored at 5˚C. All admixtures were examined visually, spectrophotometricaly as well as pH from day of preparation, 1, 3, 5 and day 7. The visual examination indicated no any existence of turbidity, precipitation and color variation. So, our admixtures ascertained physically stable. As concerned the pH, there was no any fluctuation was seen during storage Figure 3B. Results exhibited the mean percentage range between 99.5 to 99.6 % (Figures 3E, 3F), which was within official limits (>90%). The mean percentage recovery was 99.3 – 99.6% within official limits. Throughout the study, the lowered confidence-limit of the expected regression line of concentration/time outlines persisted above 90% of the initial concentrations of admixture in case of non-retain ability the formation of ppt, turbidity and cholor changes could be observed.

CONCLUSION

The stability parameters of admixture of Tramadol and Acetaminophen were evaluated under specified conditions and found stable and compatible. The storage of intravenous admixtures at low temperature (5˚C) was found to be more constant and stable than at room temperature. In addition, the stability profile of Tramadol was found superior than Acetaminophen. As one week storage of admixture, both Acetaminophen and Tramadol was lingered stable therefore could be prepared in advance (bulk) for the use in hospital by sterile manufacturing department. The physical stability of nearly all admixtures was remained stable except 02 which exhibited miner color change at 5th day (room temperature). However, strength of drugs in all admixtures was within limits (> 90%). It is believed that our study will play a significant role in formulating and storing of ready-to-use admixture of Tramadol/Acetaminophen in hospital pharmacies. In conclusion, drug admixtures can be prepared, organized and stored in advance. Conversely, it is suggested to store the drug admixture at 8˚ – 15˚ C rather than at room temperature. Moreover, stability data of these drugs could be employed in further investigations on intravenous admixtures.

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