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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING TLC-DENSITOMETRY METHOD FOR THE SIMULTANEOUS DETERMINATION OF EPERISONE HYDROCHLORIDE AND PARACETAMOL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
A rapid and reproducible stability indicating TLC-densitometric method was developed for the determination of eperisone hydrochloride and paracetamol in presence of their degraded products in bulk drugs and pharmaceutical formulations. Uniform degradation conditions were maintained by refluxing reaction mixtures for 8 h at 60°C including acidic, alkaline hydrolysis. Oxidation at room temperature, photochemical and dry heating degradation studies were also carried out. A sensitive and robust stability indicating TLC-densitometric method for simultaneous quantification of eperisone hydrochloride and paracetamol in bulk drugs and pharmaceutical formulations has been developed and validated. Separation was done on TLC aluminum sheets, pre-coated with silica gel 60F-254 using ethyl acetate: toluene: methanol (2:2:1 *v/v/v*). Spots at Rf 0.42 ± 0.04 and Rf 0.60 ± 0.02 were recognized as paracetamol and eperisone hydrochloride, respectively. Densitometric analysis of chromatoplates was carried out in absorbance mode at isobastic point 260 nm. The developed method was optimized and validated as per ICH guidelines. Method was found linear over the concentration range of 100-350 ng / spot for eperisone hydrochloride and 600-2100 ng / spot for paracetamol with the correlation coefficient (r^2) of 0.999 and 0.999 for eperisone hydrochloride and paracetamol, respectively. The developed TLC method can be applied for routine analysis of eperisone hydrochloride and paracetamol in presence of their degraded products in their combined pharmaceutical formulations.
Keywords: HPTLC, eperisone hydrochloride, paracetamol, ICH guidelines, Stability-indicating TLC-densitometry.

INTRODUCTION

Eperisone hydrochloride (1) is an antispasmodic drug which elicit skeletal muscle relaxant and vasodilator actions due to such properties in the central nervous system, effect on vascular smooth muscles and gives a variety of pharmacological effects like cervical spondylosis, headache and low back pain etc.[1,2]. Paracetamol (2), is a non-opioid, non-salicylate analgesic with an unclear mechanism of action[3]. These combinations are available in different formulations including Tablet and Capsule However, this combination is not official with British Pharmacopoeia or US Pharmacopoeia. Many analytical methods have been reported like HPLC / MS, GC / MS for the determination of eperisone hydrochloride in human plasma and pharmaceutical preparations[4-7]. HPLC and HPTLC methods are also reported for the estimation of Paracetamol in human plasma, urine, and pharmaceutical preparation[8-11]. Previously there are few HPLC and UV-Spectrophotometric methods are also reported for simultaneous estimation of eperisone hydrochloride and paracetamol in synthetic mixture and pharmaceutical preparation[12-15]. No report for the simultaneous determination of eperisone hydrochloride and paracetamol in the presence of their degraded products through TLCdensitometry has been found so far. The consideration and attention to be focus on the development of TLC-densitometry stability-indicating assay as it is fast, reliable and accurate and applicable for various simultaneous analysis of many samples with even small quantity of mobile phase, thus minimizing analysis time and cost analysis. Stress testing studies provides evidence that how the quality of a drug substance varies with time under the influence of various environmental factors (temperature, light, humidity, etc.) and helps to establish shelf life and recommended storage conditions for the drug[18]. Taking ICH guidelines Q2 (R1) in consideration, present study describes a simple and a validated TLC-densitometry method[19] for the simultaneous determination of eperisone hydrochloride and paracetamol in presence of their degraded products formed under the applied stress conditions. As all the pharmaceutical products are supposed to be assayed for potency, a validated TLC-densitometry method, demonstrating no interferences of degraded products with the drug active components can be useful in measuring these components in routine analysis.

MATERIALS AND METHODS

Standards, eperisone hydrochloride (1) and paracetamol (2), (Figure 1), were complementarily providedas gift sample by Macledos Pharmaceuticals (Pvt.) Ltd, Mumbai, India. Pharmaceutical products containing eperisone hydrochloride and paracetamol in combination (Mysone Plus Tablet) were purchased from local pharmacy shop, Dhule, Maharashtra, India. Methanol, Acetone, Ethyl Acetate of analytical grade were purchased from the Merk Specialities (Pvt.) Ltd, Mumbai, India. Sodium hydroxide was purchased from Loba Chemie, Mumbai, India. While hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2, 30 % v/v) were obtained from Fisher Scientific (UK).

Instrumentation and Chromatographic System

Stability studies were performed using reflux condenser. Planar chromatography was performed by spotting the sample on TLC aluminum plate, pre-coated with silica gel 60F-254 (10 × 10 cm) with the aid of CAMAG microliter sample syringe using CAMAG automatic TLC Linomat V

applicator (Muntenz, Switzerland). A constant sample application rate of 0.1 µL / s was adopted and the distance between the two bands was 6 mm. 5 mL of mobile phase (ethyl acetate: toluene: methanol, 2:2:1 *v/v/v*) was used for linear ascending development and chromatogram was allowed to move to a distance of 8 cm, in twin trough glass chamber (CAMAG). The chamber saturation time for mobile phase was 15 minutes at 25 ± 2°C with relative humidity 42 ± 5 %. The developed TLC plate was air dried for 15 minutes. Densitometric scanning was performed on CAMAG Reprostar scanner III in the reflectance absorbance mode at isobastic point 266 nm by utilizing D2 and Hg lamp as the source of radiation. Quantitative evaluation was performed via peak areas by WinCats software (version 1.3.0). Densitometric scanning parameters were as follows: bandwidth: 6 mm, slit width: 0.45 mm, slit length: 6 mm, scanning speed: 10 mm / s.

Preparation of Standard Solutions and Pharmaceutical Samples

Two stock solutions were prepared by dissolving 10 mg of each eperisone hydrochloride and paracetamol in 10 mL methanol, individually and in combination. Working standard solutions were prepared by dilution of stock solution with methanol to give solutions in concentration range of 100-350 µg / mL for calibration curve of eperisone hydrochloride and 600-2100 µg / mL for paracetamol. For sample preparation, Ten tablets (50 mg eperisone hydrochloride and 325 mg paracetamol each) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask and 50 mL methanol was added. After ultrasonic vibration for 10 minutes, the mixture was diluted to volume with methanol and filtered through Whatman filter paper (No. 41). 2 µL of each sample was applied on TLC plate for chromatographic analysis.

Method Validation

The developed method was validated as per the requirements of the ICH guidelines. Linearity was evaluated by determining six standard working solutions at a concentration 100-350 ng / spot for eperisone hydrochloride and 600-2100 ng / spot for paracetamol. Peak area and concentration was subjected to the least square linear regression equation to calculate the regression data and correlation coefficients. In order to calculate S / N ratio for LOD and LOQ, the formulae used were 3.3 δ / S and 10 δ / S, respectively where δ is the residual error and S stands for slope of calibration curve. In order to check the robustness, following parameters were deliberately changed within the range of ± 5 % at concentration levels (200 ng for eperisone hydrochloride and 1200 ng for paracetamol); amount of mobile phase, mobile phase composition, chamber saturation time. Intra-day and inter-day precisions were determined with the standard. For method repeatability, assay at concentration levels (200 ng and 1200 ng) was repeatedly performed six times on the same day (intra-day). For reproducibility, same samples at concentration levels (200 ng and 1200 ng) were analyzed in different days (inter-day) and results were statistically evaluated in terms of % R.S.D. For recovery studies, pre analyzed pharmaceutical drugs containing eperisone hydrochloride and paracetamol both in combination were spiked with extra 80, 100 and 120 % of eperisone hydrochloride and paracetamol. The specificity of the proposed method was analyzed by overlapping the densitogram of the standard and samples and comparing it at peak start, peak apex and peak end positions.

Preparation of Forced Degradation Products

Stability testing was carried out using different ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses. All stress studies were by refluxing the reaction mixtures for eight hours at 60°C.

Acid and Base Degradation Studies

The 10 mg of both eperisone hydrochloride and paracetamol was separately dissolved in 10 ml of methanolic solution of 1N HCl and 1N NaOH. These solutions were reflux for 8 h at 60°C temperature. The 1 ml of above solutions was taken and neutralized, then diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (2 µl i.e. 200 ng per spot for eperisone hydrochloride and 12 µl i.e. 1200 ng per spot for paracetamol) (Figure 3 and 4 respectively).

Oxidative Degradation Studies

The 10 mg of both eperisone hydrochloride and paracetamol was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (6.0 %, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on TLC plate in triplicate (2 µl i.e. 200 ng per spot for eperisone hydrochloride and 12 µl i.e. 1200 ng per spot for paracetamol) (Figure 5).

Thermal Degradation Studies

Dry heat degradation was conducted by taking standard eperisone hydrochloride and paracetamol and heated in oven at 60°C for 8 h. 10 mg of each treated standard was dissolved in 10 mL of methanol. The resultant solution was applied on TLC plate in triplicate (2 µl i.e. 200 ng per spot for eperisone hydrochloride and 12 µl i.e. 1200 ng per spot for paracetamol) (Figure 6).

Photochemical Degradation Studies

In order to evaluate photochemical degradation of eperisone hydrochloride and paracetamol, stock solution of each was directly exposed to the sunlight for three days from 8 to 18 h at 30 ± 2°C. Then, solution was applied on TLC plate in triplicate (2 µl i.e. 200 ng per spot for eperisone hydrochloride and 12 µl i.e. 1200 ng per spot for paracetamol) (Figure 7).

RESULT AND DISCUSSION

Optimization of TLC system and method validation

With the aim to develop a reliable stability indicating method, solvent system was optimized with standards, samples and degraded products. Different solvent systems of toluene, methanol, glacial acetic acid, tri-ethyl amine and ethyl acetate were tried in varying ratios. Most of the solvent systems showed diffused spots of eperisone HCl. Suitable separation with best resolution was achieved with ethyl acetate: toluene: methanol (2:2:1 *v/v/v*) which showed sharp bands with R_f value of paracetamol at 0.42 ± 0.04 and of at eperisone HCl at 0.60 ± 0.02 (Figure 2).

Table 1: Linear Regression Data and Validation Parameters of Eperisone Hcl (1) and Paracetamol (2) at 260 nm

Parameters	Eperisone HCl	Paracetamol
Linearity Range	100-350 ng / spot	600-2100 ng / spot
Correlation coefficient, r^2 ± SD	0.999 ± 1.2	0.999 ± 1.5
Slope ± SD	10.31 ± 0.6	3.58 ± 0.81
Intercept ± SD	381.88 ± .42	3365.3 ± 1.1
Y = mx + c	10.319x + 381.88	3.5813x + 3365.3
Intra-day (n = 3), % RSD	1.78	1.51
Inter-day (n = 3), % RSD	1.11	1.01
Limit of Detection	9.45	60.71
Limit of Quantification	27.66	179.11
Robustness	Robust	Robust
Specificity	Specific	Specific

Table 2: Summary of Degradation Studies of Eperisone Hydrochloride (1) and Paracetamol (2) in Combination

Degradation Condition	% Degradation of (1)	% Degradation of (2)	R_f of Degraded Product
Acidic Hydrolysis			
1 N HCl	54.7	Not Detected	0.59, 0.71, 0.78
Basic Hydrolysis			
1 N NaOH	41.61	Not Detected	0.72, 0.78
Oxidation	-	-	-
6 % H_2O_2	24.46	Not Detected	0.23, 0.5
Dry Heating	21.07	Not Detected	0.28
Photochemical Degradation	29.98	Not Detected	0.3

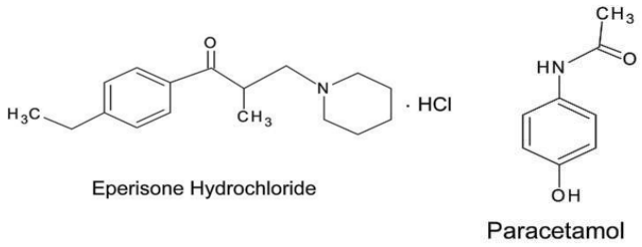

Figure 1: Structures of Eperisone HCl (1) and Paracetamol (2)

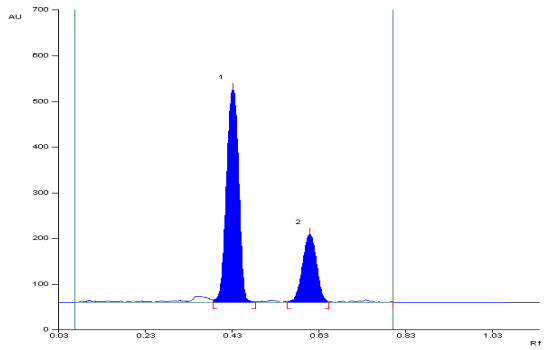

Figure 2: Chromatogram of Standard Eperisone HCl and Paracetamol in Combination at 260 nm

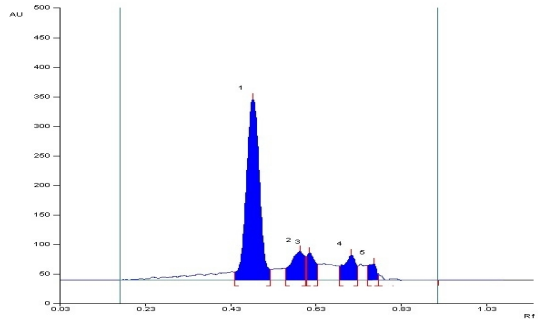

Figure 3: Eperisone HCl and Paracetamol at 260 nm in Combination Subjected to Acidic Hydrolysis (1N HCl)

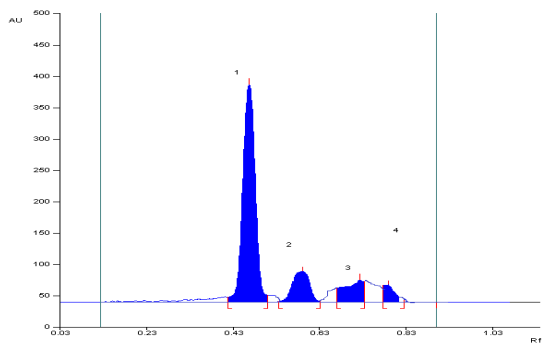

Figure 4: Eperisone HCl and Paracetamol at 260 nm in Combination Subjected to Alkaline Hydrolysis (1N NaOH

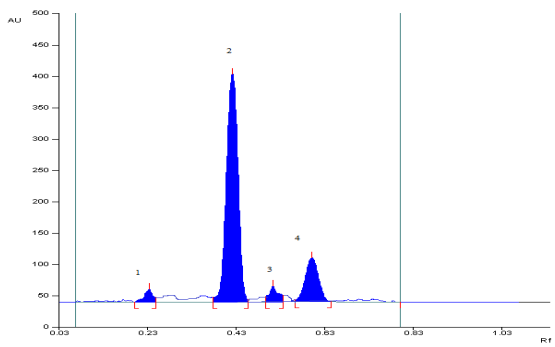

Figure 5: Eperisone HCl and Paracetamol at 260 nm in Combination Subjected to Oxidation at Room Temperature

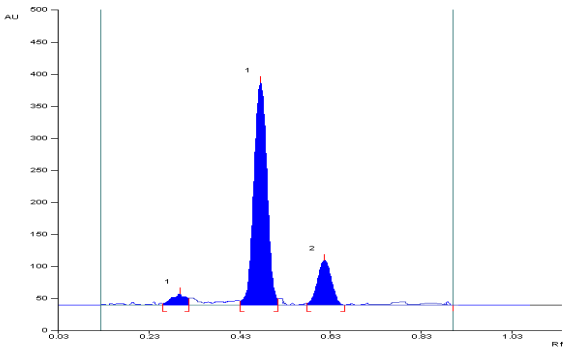

Figure 6: Eperisone HCl and Paracetamol at 260 nm in Combination Subjected to Dry Heat

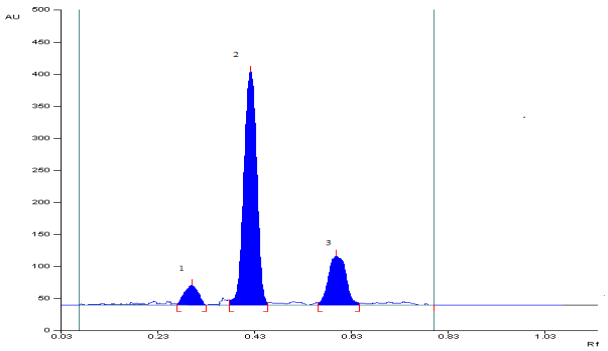

Figure 7: Eperisone HCl and Paracetamol at 260 nm in Combination Subjected to Photodegradation

Standard calibration curve for both eperisone hydrochloride and paracetamol in the concentration range of 100-350 ng and 600-2100 respectively was found linear with r2 ± S.D. 0.999 ± 1.2 and 0.999 ± 1.5, respectively. For Intra-day and interday precision, % R.S.D. observed for eperisone hydrochloride was 1.78 and 1.11, respectively while for paracetamol, 1.51 and 1.01, respectively. For eperisone hydrochloride and paracetamol, LODs were found to be 9.45 and 60.71 ng / µL, respectively while LOQs were found to be 27.66 and 179.11 ng / µL, respectively. For robustness analysis, the S.D. of peak area of standard levels (200 ng for Eperisone hydrochloride and 1200 ng for paracetamol) was estimated for each parameter. S.D. was 35.11 and 22.71 for changing the amount of mobile phase, 17.59 and 32.31 for varying in mobile phase composition and 19.76 and 46.10 for varying chamber saturation time for eperisone hydrochloride and paracetamol, respectively. The linear regression data and the method validation results are summarized in Table 1.

Stability Indicating Property of Eperisone HCl and Paracetamol in Combination

A combined standards solution showed three additional peaks under acidic (1N HCl) condition at R_f 0.59, 0.71 and 0.78 while paracetamol remains unaffected. Moreover, under acidic conditions, Eperisone HCl was 45.3 % recovered. Under alkaline conditions, Eperisone HCl were 58.39 % recovered. Two common additional peaks were generated under all alkaline (1N NaOH) conditions at R_f 0.72 and 0.78 while paracetamol remains unaffected. For oxidation reaction, under oxidation condition (reaction mixture kept for 8 h at room temperature). Eperisone HCl shows two additional peaks at R_f 0.23 and 0.5. Under photochemical degradation shows one additional peak at R_f 0.3 and thermal (dry heat) condition shows one additional peak at R_f 0.28. Stress degradation study of Eperisone HCl and paracetamol in combined sample is summarized in Table 2.

CONCLUSION

A validated Densitometric / TLC method for routine analysis to determine the stability of Eperisone hydrochloride and paracetamol in pharmaceutical dosage forms has been developed. Present study demonstrates the degradation susceptibility of drugs to different stress conditions and thus helps in determining the changes in chemical and physical properties of the drug samples with time. It also helps in understanding the mechanism and pathway of degraded products formation and in developing a profile reflecting the changes in identity, purity and potency of the product. The developed stability indicating Densitometric / TLC method is simple, reproducible and can be used for the simultaneous analysis of two active components (Eperisone hydrochloride and paracetamol). It appears suitable for routine analysis of these components selectively in presence of their degraded products in combined pharmaceutical formulations.

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