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Simultaneous Determination of Acetaminophen, Pamabrom and Pyrilamine Maleate in Pharmaceutical Formulations Using Stability Indicating HPLC Assay Method

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Abstract. A simple, specific and accurate stability indicating RP-HPLC method was developed for the determination of acetaminophen, pamabrom and pyrilamine maleate simultaneously in pharmaceutical dosage forms. Successful separation of all the components was enacted within 10 min using C18 column with mobile phase of methanol and acidified water (pH 1.8) in the ratio of (27: 73 v/v respectively). Flow rate of the mobile phase was 1.5 mL/min with detection at 300 nm. The method was validated in accordance with ICH guidelines. Response was a linear function of concentration over the range of 50- 150 µg/mL for acetaminophen, 2.5-7.5 µg/mL for pamabrom and 1.5-4.5 µg/mL for pyrilamine maleate. The method resulted in excellent separation of all the analytes along with their stress induced degradation products with acceptable peak tailing and good resolution. It is therefore can be applied successfully for simultaneous determination of acetaminophen, pamabrom and pyrilamine maleate in pharmaceutical formulations and their stability studies.

Key words: RP-HPLC, Acetaminophen, Pamabrom, Pyrilamine, ICH guidelines.

Resumen. Se ha desarrollado un procedimiento simple y específico para la determinación de acetaminofeno, pamabrom y maleato de pirilamina en formulaciones farmacéuticas por HPLC en fase inversa. La separación de todos los componentes se obtuvo en 10 min utilizando una columna C18 y la fase móvil compuesta por metanol y solución acuosa a pH 1.8 (con ácido sulfúrico) en relación 27: 73 v/v. La velocidad del flujo en la columna fue de 1.5 mL/min y la detección espectrofotométrica en 300 nm. El método fue validado siguiendo los criterios ICH. La respuesta fue lineal en el intervalo de concentraciones 50- 150 µg/mL para acetaminofeno, 2.5-7.5 µg/mL para pamabrom y 1.5-4.5 µg/mL para maleato de pirilamina. El procedimiento permitió la separación de los tres analitos y de sus productos de degradación inducidos en condiciones de estrés con aceptable simetría de picos y resolución cromatográfica. Es por ello que este procedimiento puede ser empleado para la determinación de acetaminofeno, pamabrom y maleato de pirilamina en formulaciones farmacéuticas y en estudios de su estabilidad.

Palabras clave: Cromatografía de líquidos de alta resolución en fase inversa (RP-HPLC), Acetaminofeno, Pamabrom, Pirilamina, criterios ICH (Conferencia Internacional sobre Armonización de los Requisitos Técnicos para el Registro de Productos Farmacéuticos para Uso Humano).

Introduction

Acetaminophen commonly called Paracetamol is chemically designated as 4-hydroxyacetanilide (Fig. 1). It shows both analgesic and antipyretic properties and is available from the open market without prescription as well as through prescription. Different dosage forms of paracetamol such as tablet, capsules, drops etc. are available in the open market and its different combinations with other drugs have been enlisted in pharmacopeias. [1-2] Pamabrom (Fig. 2) is a common diuretic existed as 1:1 mixture of 8-Bromo-3,7-dihydro-1,3-dimethyl-1-*H*-purine-2,6-dione and 2-amino-2-methyl-1-propanol, where 8-Bromo-3,7-dihydro-1,3-dimethyl-1-*H*-purine-2,6-dione is the active diuretic agent. [3] Pyrilamine maleate (Fig. 3) having the chemical name 1,2-Ethanediamine, N-[(4-methoxyphenyl)methyl]-N', N'-dimethyl-N-2-pyridinyl-, (Z)-2-butenedioate (1:1

is an antihistamine used to reduce the allergic conditions and reduce symptoms of cold. [4]

The combination of three active ingredients acetaminophen, pamabrom and pyrilamine is used for the treatment of symptoms of mild to moderate premenstrual syndrome in addition to additive effects as analgesic as well as antihistamine and mild diuretic effects.[5] This combination is available in different strengths in different dosage form with various brand names. Most common are the tablet dosage form having strength of 500mg, 25mg and 15mg respectively of Acetaminophen, Pamabrom and Pyrilamine.

Literature review did not provide any analytical method for the simultaneous determination of above three components. Individual search of the three components regarding their analytical methods revealed a flood of HPLC methods for acetaminophen either individually or in combination with other

active ingredients. [6-17] The search of the other two components viz. pamabrom and pyrilamine resulted in only few analytical methods. [18-21]

The fixed dose combination containing acetaminophen, pamabrom and pyrilamine maleate is available in the market in many countries yet no official pharmacopoeia has adopted that combination. Literature review also resulted in failure of any reported HPLC method for the simultaneous determination of these three drugs in fixed dose combination. Therefore efforts were done to develop and validate RP-HPLC method for simultaneous determination of these drugs and their stress induced degradation products in pharmaceutical formulations. The described method is able to separate all three drugs from the stress induced degradation within 10 min, so it can be used for stability studies also.

Results and discussion

Method Development and Optimization

The main objective of this research work was to achieve the best conditions for separation of acetaminophen, pamabrom and pyrilamine maleate simultaneously in their fixed dose combination.

Initially various mobile and stationary phases were tried to accomplish the best separation conditions and resolution be-

tween acetaminophen, pamabrom and pyrilamine maleate. For the initial trial, Hypersil BDS C8 column was chosen with mobile phase consisting of 1 M Potassium dihydrogen phosphate and acetonitrile in different ratios (50 : 50, 40 : 60, 30 : 70, 20 : 80). Under all these condition elution of acetaminophen occurred only and there was no separation between pamabrom and pyrilamine maleate.

Then stationary phase was switched to Cyano column using same ratios of 1 M Potassium dihydrogen phosphate and acetonitrile as they were used with Hypersil BDS C8 column. Here also the elution of acetaminophen occurred with good peak shape but remaining two peaks remain merged with each other. pH of 1 M Potassium dihydrogen phosphate was varied from 2 - 7 in order to separate the merged components but all attempts remained fruitless.

Further trials were carried out using Promosil C18 column but result was the same as for other two columns. Then mobile phase was changed from buffer to acidified water and methanol was used instead of acetonitrile with different concentrations (50:50, 40:60, 30:70, 20:80) and with different pH values of acidified water (1.5 - 4.5). The trick works here and all the three components were eluted. At last, mobile phase of acidified water (pH 1.8) and methanol in the ratio of 73: 27 was selected that provides best separation conditions for the mentioned three components along with C18 column. Under these conditions tailing of all the components was less than 1.5 with retention times of 2.0, 2.8 and 7.5 minutes for acetaminophen, pamabrom and pyrilamine maleate at a flow rate of 1.5 ml per minute.

Analytical method validation

The developed analytical method was validated using ICH guidelines. [22] Linearity, accuracy, precision, robustness, specificity, stability of solutions and limit of detection and quantitation were performed.

Linear calibration plots of the proposed method were obtained over concentration ranges of 50-150 µg/mL (50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 µg/mL) for acetaminophen, 2.5-7.5 µg/mL for pamabrom (2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 µg/mL) and 1.5-4.5 µg/mL for pyrilamine maleate (1.5, 1.8, 2.1, 2.4, 2.7, 3.0, 3.3, 3.6, 3.9, 4.2, 4.5). The linear regression equation for acetaminophen was $Y = 12039X + 401076$ with correlation coefficient of 0.9993. For pamabrom, it was $Y = 1246.9X + 30914$ with correlation coefficient of 0.9993 and for pyrilamine maleate it was $Y = 1579.5X + 38151$ with correlation coefficient of 0.9995. The graphics of linearity are shown in Figs. 4-6.

The limit of detection (LOD) and quantitation (LOQ) were determined by making serials of dilutions. LOD was found to be 0.23 µg/mL for acetaminophen, 0.09 µg/mL for pamabrom and 0.26 µg/mL for pyrilamine maleate respectively (signal to noise ratio of 3: 1). LOQ was found to be 0.77 µg/mL for acetaminophen, 0.30 µg/mL for pamabrom and 0.87 µg/mL pyrilamine maleate respectively (signal to noise ratio of 10: 1).

Accuracy of the method was evaluated in triplicates using standard addition technique at three concentration levels i.e.

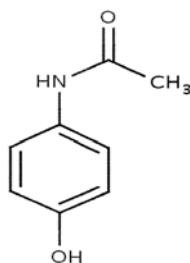


Fig. 1. Chemical Structure of Acetaminophen

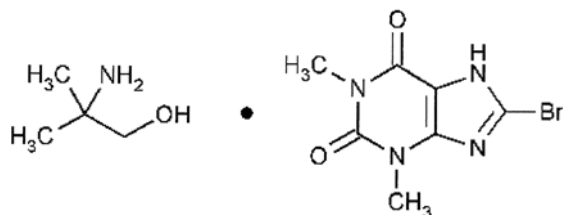


Fig. 2. Chemical Structure of Pamabrom

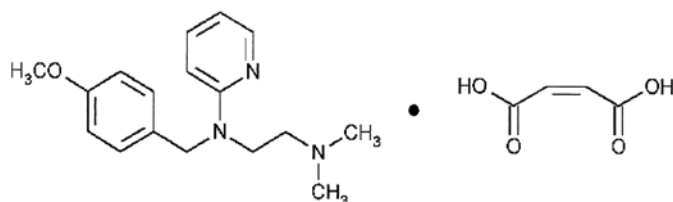


Fig. 3. Chemical Structure of Pyrilamine Maleate

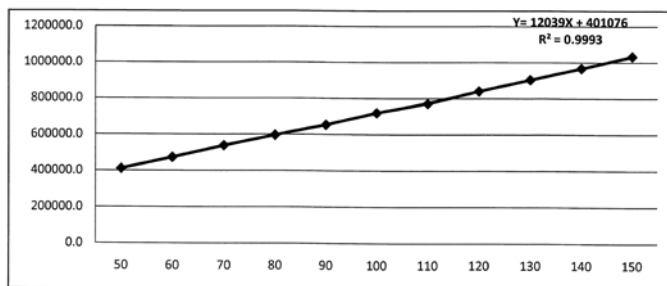


Fig. 4. Linearity graph for acetaminophen

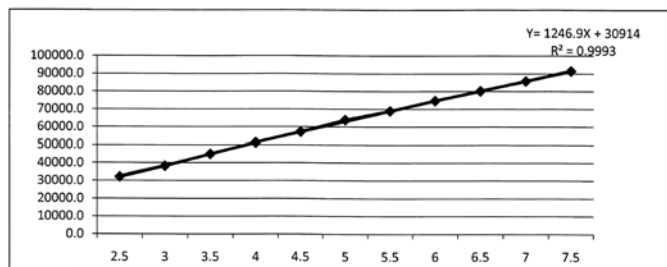


Fig. 5. Linearity graph for pamabrom

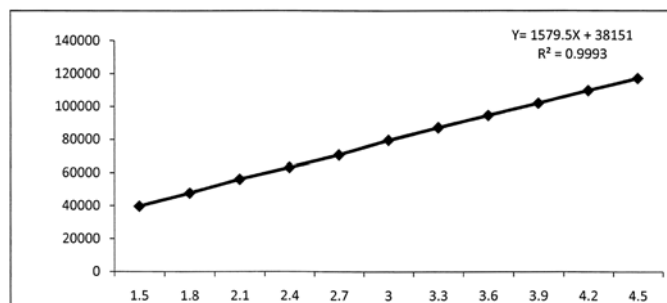


Fig. 6. Linearity graph for pyrilamine maleate

80 %, 100 % and 120 % of target test concentration (100 µg/mL of acetaminophen, 5 µg/mL of pamabrom & 3 µg/mL of pyrilamine maleate). Percentage recoveries along with standard deviation and relative standard deviations for each analyte are given in Table 1. Recovery studies showed the method to be highly accurate and suitable for intended use.

For intra-day precision, three set of concentrations of acetaminophen, pamabrom and pyrilamine maleate were tested six times within the same day. For intermediate precision, two different analysts from the same Laboratory tested the same three concentrations six times. Relative standard deviation (RSD %) of the peak area was then calculated. The results of intra-day and inter-day precision are presented in Table 2.

For carrying out the robustness, very small changes were carried out in mobile phase composition, flow rate and pH of acidified water. The results (Assay, tailing factor, theoretical plates and resolution) showed that slight variations in chromatographic conditions had negligible effect on the chromatographic parameters. All the chromatographic parameters remained within the acceptable criteria as described earlier. It was thus concluded that the method is robust for the intended use.

Specificity of the developed method was evaluated by applying different stress conditions (acid, base, oxidation, thermal, humidity and photolytic) to acetaminophen, pamabrom and pyrilamine maleate in combination form. From the result of forced degradation studies, it is clear that all the three components remain intact under heat stress and humid conditions. In acidic conditions, acetaminophen and pyrilamine maleate were degraded up to 4 % whereas no degradation was observed for pamabrom. Basic stress caused the degradation of acetaminophen and pyrilamine up to 12.5 and 13.8 % respectively. Remarkable degradation was observed in case of pamabrom and pyrilamine maleate in oxidative conditions, where pamabrom and pyrilamine maleate were degraded up to 10.6 % and

Table 1. Accuracy of the Proposed HPLC Method

Drugs	Spiked Concentration (µg mL ⁻¹)	Recovery (%)	SD	RSD (%)
Acetaminophen	80.0	102.7	0.28	0.27
	100.0	100.8	0.09	0.09
	120.0	98.3	0.15	0.15
Pamabrom	4.0	99.3	0.39	0.39
	5.0	101.7	0.37	0.36
	6.0	101.3	0.21	0.21
Pyrilamine	2.4	101.6	0.46	0.45
	3.0	100.1	0.41	0.41
	3.6	103.1	0.18	0.17

Table 2. Intra-Day and Intermediate Precision of the Proposed HPLC Method

Ingredient	n	Repeatability ± RSD (%)	Intermediate Precision ± RSD (%)
Acetaminophen	18	0.06	0.13
Pamabrom	18	0.56	0.71
Pyrilamine	18	0.88	1.13

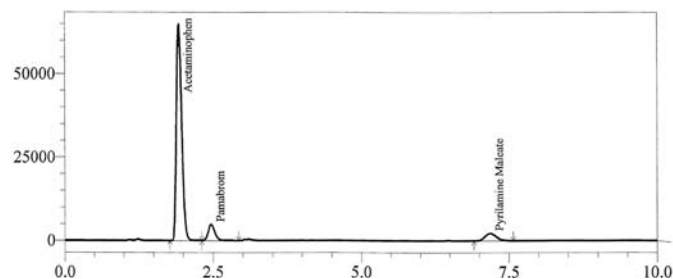


Fig. 7. Chromatogram of acetaminophen, pamabrom and pyrilamine maleate standard

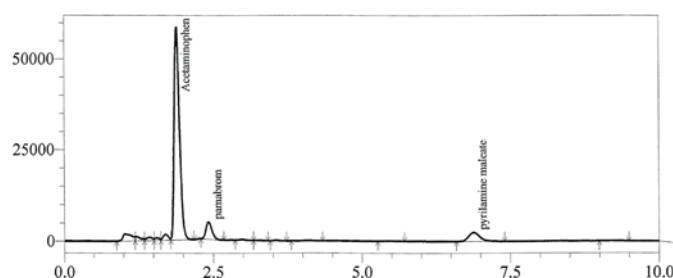


Fig. 8. Chromatogram of acetaminophen, pamabrom and pyrilamine maleate under basic stress

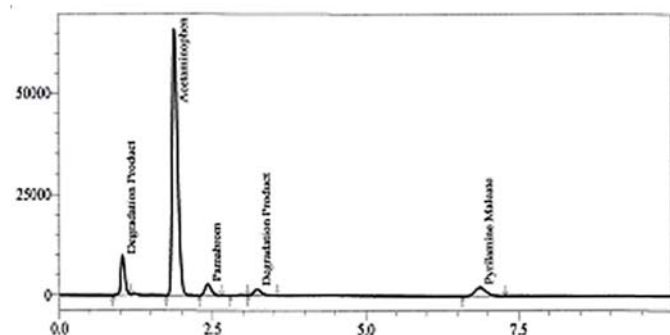


Fig. 9. Chromatogram of acetaminophen, pamabrom and pyrilamine maleate under oxidative stress

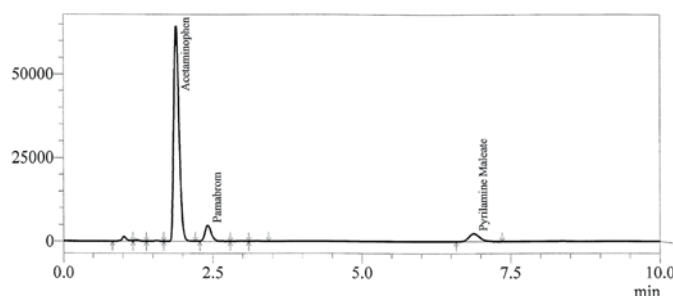


Fig. 10. Chromatogram of acetaminophen, pamabrom and pyrilamine maleate under acidic stress

44 % respectively. The amount degraded was calculated by subtracting the recovered amount in each stress condition from the recovered amount of un-stressed samples. The representative chromatograms under all the stress conditions are shown in Figs. 7-10.

In addition to the percentage degradation of each drug, a number of degradation products (impurities) were produced under acidic, oxidative and photolytic stress conditions. The stress induced degradation products (impurities) were unique to acidic (7.93, 9.47 min), oxidative (3.82, 6.16, and 10.82 min) and photolytic (5.10, 6.09, 7.17, and 8.45) stress conditions. The data is shown in Table 3.

Application of the Method

Application of the method was checked by analyzing the acetaminophen, pamabrom and pyrilamine maleate in commercially available pharmaceutical products. The results are provided in Table 4 which showed high percentage recoveries and low RSD (%) values for both analytes.

Experimental

Chemicals and Reagents

Reference standards of acetaminophen, pamabrom and pyrilamine maleate with stated purity of 98.81, 99.12 and 99.20 % respectively were obtained from CCL Pharmaceuticals (Lahore, Pakistan). Femistar tablet claimed to contain 500 mg per tablets of acetaminophen, 25 mg per tablets of pamabrom and 15 mg per tablet of pyrilamine maleate were used in this study. Methanol (HPLC grade), sulphuric Acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide (analytical reagent grade) were of Fluka and were purchased from their local agent in Lahore, Pakistan. Mobile phase was filtered using 0.45 μ m nylon filters by Millipore (USA).

Equipment and Chromatographic Conditions

HPLC apparatus consisted of Shimadzu LC-20A system (Kyoto, Japan) with auto sampler and UV detector set at 300 nm. BDS Promosil C18 column (250 \times 4.6 mm, 5 μ m particle size) was used for carrying out all the experimental work. Acidified water (pH 1.8) and methanol in the ratio of (73: 27 v/v respectively) were used as mobile phase at a flow rate of 1.5 mL/min. All chromatographic experiments were performed at room temperature (25 $^{\circ}$ C \pm 2 $^{\circ}$ C).

Preparation of Mobile Phase

Mobile phase was prepared by mixing acidified water (pH 1.8) and methanol in the ratio of (73: 27 v/v respectively). Acidified water was prepared using sulphuric acid and then dilution to set pH 1.8 with water.

Preparation of Standard Solution

500 mg acetaminophen, 25 mg of pamabrom and 15 mg of pyrilamine maleate were accurately weighed in 100 ml flask, dissolved and then diluted with mobile phase. This solution was then further diluted to get the desired concentrations.

Table 3. Stress Testing Results of Acetaminophen, Pamabrom, Pyrilamine Maleate

Nature of stress	Recovery of Acetaminophen \pm RSD (%)	Recovery of Pamabrom \pm RSD (%)	Recovery of Pyrilamine \pm RSD (%)
Heat stress	100.60 \pm 0.22	98.59 \pm 0.22	97.41 \pm 2.14
1N HCl	95.97 \pm 0.08	99.62 \pm 0.84	95.47 \pm 1.31
1N NaOH	87.41 \pm 0.15	100.42 \pm 0.29	86.22 \pm 0.69
10 % H ₂ O ₂	97.99 \pm 0.03	89.40 \pm 0.44	56.04 \pm 2.22
Humidity & Light	100.49 \pm 0.06	98.94 \pm 0.77	99.66 \pm 1.08

Table 4. Assay results of acetaminophen, pamabrom and pyrilamine maleate in commercial tablets

Product	Ingredient	Label Value (mg per Tablet)	Found (mg)	Recovery \pm RSD (%)
Femistar	Acetaminophen	500	502.0	100.4 \pm 0.21
	Pamabrom	25	24.96	99.84 \pm 0.75
	Pyrilamine	15	15.04	101.33 \pm 0.88

* n = Average of 10 determinations

Linearity

Linear calibration plots of the proposed method were obtained by analyzing eleven solutions over concentration ranges of 50-150 $\mu\text{g/mL}$ (50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 $\mu\text{g/mL}$) for acetaminophen, 2.5-7.5 $\mu\text{g/mL}$ for pamabrom (2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 $\mu\text{g/mL}$) and 1.5-4.5 $\mu\text{g/mL}$ for pyrilamine maleate (1.5, 1.8, 2.1, 2.4, 2.7, 3.0, 3.3, 3.6, 3.9, 4.2 and 4.5 $\mu\text{g/mL}$). Each solution was prepared in triplicate. The acceptance criterion was the value of correlation coefficient which should be close to 1.

Accuracy

Accuracy of the method was evaluated in triplicates using standard addition technique at three concentration levels i.e. 80 %, 100 % and 120 % of target test concentration (100 $\mu\text{g/mL}$ of acetaminophen, 5 $\mu\text{g/mL}$ of pamabrom & 3 $\mu\text{g/mL}$ of pyrilamine maleate). The recoveries of analytes were then calculated. Generally acceptable values for accuracy ranges from 95-105 %.

Precision

For intra-day precision, three set of concentrations of acetaminophen, pamabrom and pyrilamine maleate were tested six times within the same day. From these replicates, percentage RSD was calculated. For intermediate precision, two different analysts from the same Laboratory tested the same three concentrations six times. RSD was then calculated from those. RSD less than 2 % is generally accepted for precision so this criterion was set as acceptable for this study.

Specificity

To demonstrate the stability indicating properties of the proposed method, accelerated degradation studies were performed on acetaminophen, pamabrom and pyrilamine in

tablet dosage form by applying different stress conditions. The stress conditions employed include light & humidity exposure, heat (60 $^{\circ}\text{C}$), acid (1N HCl), base (1N NaOH), and Oxidative (10% H₂O₂) stress. The monitoring time was 24 hours for acid (1N HCl), base (1N NaOH), oxidative (10% H₂O₂), light exposure and humidity stress and 60 minutes of heat stress (60 $^{\circ}\text{C}$).

Robustness

For carrying out the robustness, very small changes were carried out in mobile phase composition, flow rate and pH of acidified water. The effect of these small changes on retention time, tailing factor, resolution and number of theoretical plates of each analyte was then assessed. Tailing factors less than 1.5, theoretical plates greater than 2000 and resolution greater than 1.5 was acceptable criteria for the proposed method.

Solution stability and Mobile Phase stability

To check the stability of all the three active components of this ternary combination in solution form, the solution of these components was placed in tight containers at room temperature for 48 hours and their stability was checked after each 12 hours period. Mobile phase stability was also checked by using 12-48 hour old mobile phase for the preparation of analyte solution and then calculating the recovery of the active components in that mobile phase solution.

Application of the method in tablets

20 tablets were weighed and the average weight was calculated. These tablets were then ground to fine powder. Weight of the powder equivalent to mean weight of one tablet of Femistar (composition 500 mg acetaminophen, 25 mg of pamabrom and 15 mg of pyrilamine maleate per tablet) was diluted to 100 mL

with mobile phase. 2 ml of this solution was then diluted to 100 mL with mobile phase to obtain concentration equal to 100 µg/mL of acetaminophen, 5 µg/mL of pamabrom and 3 µg/mL of pyrilamine maleate. Percentage recovery of each analyte was then calculated using the developed method. Percentage recovery from 95-105 % was considered as acceptable for this study.

Conclusions

A simple, sensitive, isocratic and accurate reverse phase HPLC method has been described for simultaneous determination of acetaminophen, pyrilamine maleate and pamabrom in pharmaceutical formulations. The proposed method was validated by testing its linearity, accuracy, and precision, limits of detection and quantitation and specificity. The method was good enough to separate the peaks of active pharmaceutical ingredients (APIs) from the degradation products (produced during forced degradation studies). It is also clear from the chromatograms that both the active ingredient peaks in all the stress conditions were free from any sort of degradation impurities. All these convince us to conclude that the method can be successfully used for any sort of stability and validation studies.

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